

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
10 May 2001 (10.05.2001)

PCT

(10) International Publication Number
WO 01/32153 A2

(51) International Patent Classification⁷: **A61K 31/00**

(21) International Application Number: **PCT/CA00/01316**

(22) International Filing Date:
3 November 2000 (03.11.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/163,405 4 November 1999 (04.11.1999) US
60/163,394 4 November 1999 (04.11.1999) US

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

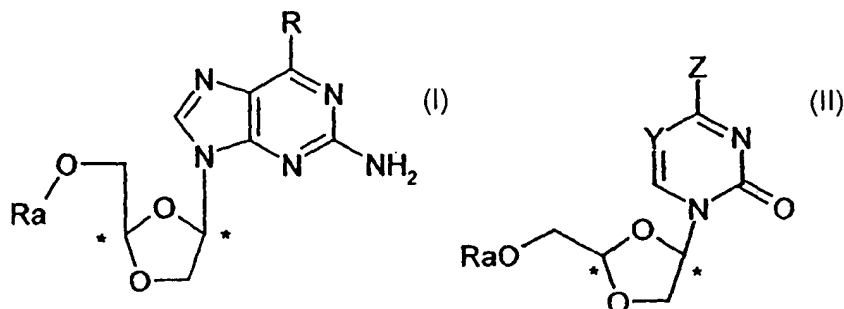
(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— Without international search report and to be republished
upon receipt of that report.

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: METHOD FOR THE TREATMENT OR PREVENTION OF *FLAVIVIRIDAE* VIRAL INFECTION USING NUCLEO-
SIDE ANALOGUES



(57) Abstract: In accordance with the present invention there is provided a method for treating or preventing a *Flaviviridea* viral infection in a host comprising administering a therapeutically effective amount of at least one compound of formula (I) or (II) or a pharmaceutically acceptable salt thereof, wherein Ra, R, Z and Y are defined in the application.

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METHOD FOR THE TREATMENT OR PREVENTION OF FLAVIVIRIDAE VIRAL
INFECTION USING NUCLEOSIDE ANALOGUES

FIELD OF THE INVENTION

The present invention relates to a method for the treatment or prevention *Flaviviridae* viral infection using nucleoside analogues.

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BACKGROUND OF THE INVENTION

Hepatitis is a disease occurring throughout the world. It is generally of viral nature, although there are other causes known. Viral hepatitis is by far the most common form of hepatitis. Nearly 750,000 Americans are affected by hepatitis each year, and out of those, more than 150,000 are infected with the hepatitis C virus (HCV).

20

HCV is a positive-stranded RNA virus belonging to the *Flaviviridae* family and has closest relationship to the pestiviruses that include hog cholera virus and bovine viral diarrhea virus (BVDV). HCV is believed to replicate through the production of a complementary negative-strand RNA template. Due to the lack of efficient culture replication system for the virus, HCV particles were isolated from pooled human plasma and shown, by electron microscopy, to have a diameter of about 50-60 nm. The HCV genome is a single-stranded, positive-sense RNA of about 9,600 bp coding for a polyprotein of 3009-3030 amino-acids, which is cleaved, post-translationally by cellular and two viral proteinases into
30 mature viral proteins (core, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B). It is believed that the structural proteins, E1 and E2, the major glycoproteins are embedded into a viral lipid envelop and form stable heterodimers. The structural core protein interacts with the viral RNA genome to form the nucleocapsid. The genes or regions designated

NS2 to NS5 code for nonstructural proteins some of which have enzymatic functions involved in virus replication and protein processing including a polymerase, protease and helicase.

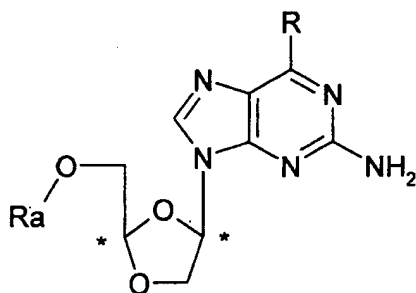
The main sources of contamination with HCV is blood. The magnitude of the HCV infection as a health problem is illustrated by the prevalence among high-risk groups. For example, 60% to 90% of hemophiliacs and more than 80% of intravenous drug abusers in western countries are chronically infected with HCV. For intravenous drug abusers, the prevalence varies from about 28% to 70% depending on the population studied. The proportion of new HCV infections associated with post-transfusion has been markedly reduced lately due to advances in diagnostic tools used to screen blood donors.

The treatment currently available for HCV infection is interferon (IFN), ribavirin, and a combinaison of the two (REBETRON). However, according to different clinical studies, only 70% of treated patients normalize alanine aminotransferase (ALT) levels in the serum and after discontinuation of IFN, 35% to 45% of these responders relapse. In general, only 20% to 25% of patients have long-term responses to IFN. On the other hand, pilot studies suggest that combination treatment with IFN plus Ribavirin (RIBA) results in sustained response in the majority of patients. Different genotypes of HCV respond differently to IFN therapy, genotype 1b is more resistant to IFN therapy than type 2 and 3.

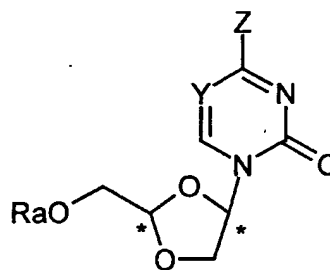
There is therefore a great need for the development of therapeutic agent for treating or preventing *Flaviviridae* viral infection.

Summary of the invention

The present invention provides a method for treating or preventing a *Flaviviridea* viral infection in a host comprising administering a therapeutically effective amount of at least one compound selected from:



(I)



(II)

10

or

or a pharmaceutically acceptable salts thereof, wherein:

R is H, $-NR_2R_3$ or OR_4 wherein

R_2 is H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_3 -cycloalkyl;

R_3 is H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl;

R_4 is H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl; and

20 Z is H, OR_4' , or $-NR_2'R_3'$ wherein:

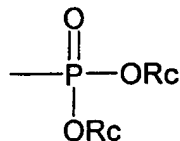
R_2' is H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_3 -cycloalkyl,

R_3' is H, C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl;

R_4' is H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl; and
and;

Y is N or C-X;

X is chosen from of H, halogen, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, CN, CF₃, N₃, NO₂, C₆₋₁₀ aryl, C₆₋₁₀ heteroaryl and COR_b wherein R_b is chosen from of H, OH, SH, C₁₋₆ alkyl, C₁₋₆ aminoalkyl, C₁₋₆ alkoxy and C₁₋₆ thioalkyl;
and R_a is chosen from of H, monophosphate, diphosphate, triphosphate, carbonyl substituted with a C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₆₋₁₀ aryl, and



wherein each R_c are independently chosen from H, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl and an hydroxy protecting group,

wherein said nucleoside is present in the form of the (-) enantiomer, the (+) enantiomer or mixtures thereof, including racemic mixtures.

The present invention provides a method for treating a *Flaviviridea* viral infection in a host comprising administering a therapeutically effective amount of at least one compound as described in the present application.

The present invention provides a method for preventing a *Flaviviridea* viral infection in a host comprising administering a therapeutically effective amount of at least one compound as described in the present application.

The present invention provides a method for reducing the biological activity of a *Flaviviridea* viral infection in a host comprising administering a therapeutically effective amount of at least one compound as described in the present application.

In another aspect, there is provided a pharmaceutical formulation comprising the compound of the invention in

combination with a pharmaceutically acceptable carrier or excipient.

Still another aspect, there is provided a method for treating or preventing a *Flaviviridea* viral infection in a host comprising administering to the subject a combination comprising at least one compound according to formula I or formula II and at least one further therapeutic agent.

- 10 In another aspect of the invention is the use of a compound according to formula I, for the preparation of a medicament for treating or preventing a viral infections in a host.

In another aspect of the invention is the use of a compound according to formula II, for the preparation of a medicament for treating or preventing a viral infection in a host.

DETAILED DESCRIPTION OF THE INVENTION

- 20 In one embodiment, the viral infection is chosen from *Flaviviridea* viral infections.

In one embodiment, the *Flaviviridea* viral infection is chosen from of Hepatitis C virus(HCV), bovine viral diarrhea virus(BVDV), hog cholera and yellow fever.

In an other embodiment, the *Flaviviridea* viral infection is Hepatitis C.

- 30 In one embodiment, there is also provided a method for inhibiting or reducing the activity of viral polymerase in a host, when compared to a standard comprising administering a therapeutically effective amount of a compound having the formula I.

In one embodiment, there is also provided a method for inhibiting or reducing the activity of viral polymerase in a host, when compared to a standard, comprising administering a therapeutically effective amount of a compound having the formula II.

In one embodiment, there is also provided a method for inhibiting the activity of viral polymerase in a host, when compared to a standard comprising administering a
10 therapeutically effective amount of a compound having the formula I.

In one embodiment, there is also provided a method for inhibiting the activity of viral polymerase in a host, when compared to a standard, comprising administering a therapeutically effective amount of a compound having the formula II.

In one embodiment, there is also provided a method for
20 reducing the activity of viral polymerase in a host, when compared to a standard comprising administering a therapeutically effective amount of a compound having the formula I.

In one embodiment, there is also provided a method for reducing the activity of viral polymerase in a host, when compared to a standard, comprising administering a therapeutically effective amount of a compound having the
30 formula II.

In another embodiment, the viral polymerase is HCV polymerase.

In one embodiment, the compounds and methods of the present invention comprise those wherein the following embodiments are present, either independently or in combination.

In one embodiment, R_4 is C_{1-6} alkyl.

In one embodiment, R_4 is methyl or ethyl.

In one embodiment, R is NH_2 , H or OH .

In a further embodiment R is NH_2 or OH .

In a further embodiment R is OH .

In one embodiment R is $-NR_2R_3$ wherein R_2 is C_{3-8} cycloalkyl and R_3 is H .

10 In one embodiment R is $-NR_2R_3$ wherein R_2 is C_{3-5} cycloalkyl and R_3 is H .

In one embodiment R is $-NR_2R_3$ wherein R_2 is cyclopropyl or cyclopentyl and R_3 is H .

In one embodiment R is $-NR_2R_3$ wherein R_2 is cyclopropyl and R_3 is H .

In one embodiment R_a is chosen from H , monophosphate, diphosphate, and triphosphate.

20 In one embodiment R_a is chosen from monophosphate, diphosphate, and triphosphate.

In one embodiment R_a is triphosphate.

In one embodiment R_a is H .

In one embodiment, Z is H , OH , $-NR_2R_3$ wherein:

R_2 is H , or C_{1-6} alkyl; and

R_3 is H , or C_{1-6} alkyl.

In one embodiment, R_4 is C_{1-6} alkyl.

In one embodiment, R_4 is methyl or ethyl.

30 In one embodiment Z is chosen from OH or NH_2 .

In one embodiment Z is OH .

In another embodiment Z is NH_2 .

In another embodiment, Y is N .

In another embodiment, Y is $C-X$.

In another embodiment, X is H , C_{1-6} alkyl or halogen.

In another embodiment, X is H, methyl or halogen.

In another embodiment, X is methyl, $-\text{HC}=\text{CH}_2$ and $-\text{C}\equiv\text{CH}$.

In another embodiment, X is H or halogen.

In another embodiment, X is halogen.

In another embodiment, X is H, methyl or F.

In another embodiment, X is H or F.

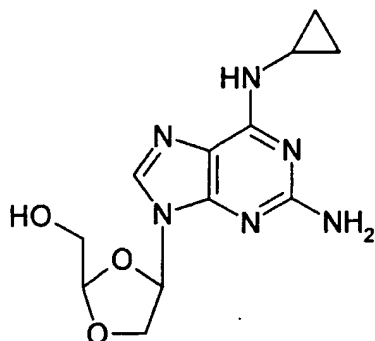
In another embodiment, X is H.

In another embodiment, X is F.

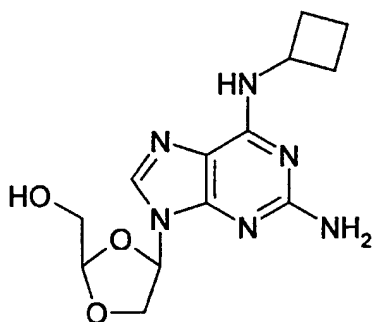
- 10 It will be appreciated by those skilled in the art that the compounds of formula (I) contain at least two chiral centers which are marked by an asterisk (*) on the general formula (I) or (II). The compounds of formula (I) and (II) thus exist in the form of two different optical isomers (i.e. (+) or (-) enantiomers or β -L and β -D). All such enantiomers and mixtures thereof including racemic mixtures are included within the scope of the invention. The single optical isomer or enantiomer can be obtained by method well known in the art, such as chiral synthesis, chiral HPLC, enzymatic
- 20 resolution and chiral auxiliary.

Compounds of the invention include, for compounds having the structure of the formula I:

- Compound 1** cis-2-hydroxymethyl-4-(2'-amino-6'-cyclopropylamino-purine-9'-yl)-1,3-dioxolane; **Compound 1(-)** (-)cis-2-hydroxymethyl-4-(2'-amino-6'-cyclopropylamino-purine-9'-yl)-1,3-dioxolane; ; **Compound 1(+)** (+)cis-2-hydroxymethyl-4-(2'-amino-6'-cyclopropylamino-purine-9'-yl)-1,3-dioxolane
- 30

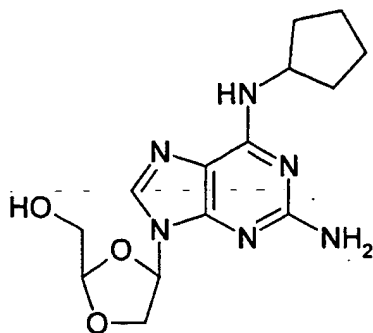


Compound 2 cis-2-hydroxymethyl-4-(2'-amino-6'-cyclobutylamino-purine-9'-yl)-1,3-dioxolane; **Compound 2(-)** (-)cis-2-hydroxymethyl-4-(2'-amino-6'-cyclobutylamino-purine-9'-yl)-1,3-dioxolane **Compound 2(+)** (+)cis-2-hydroxymethyl-4-(2'-amino-6'-cyclobutylamino-purine-9'-yl)-1,3-dioxolane

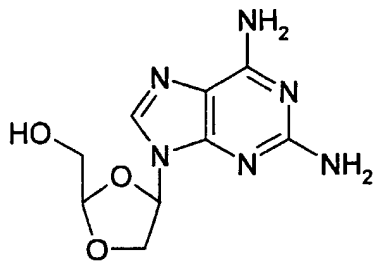


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Compound 3 cis-2-hydroxymethyl-4-(2'-amino-6'-cyclopentylamino-purine-9'-yl)-1,3-dioxolane; **Compound 3(-)** (-)cis-2-hydroxymethyl-4-(2'-amino-6'-cyclopentylamino-purine-9'-yl)-1,3-dioxolane; **Compound 3(+)** (+)cis-2-hydroxymethyl-4-(2'-amino-6'-cyclopentylamino-purine-9'-yl)-1,3-dioxolane

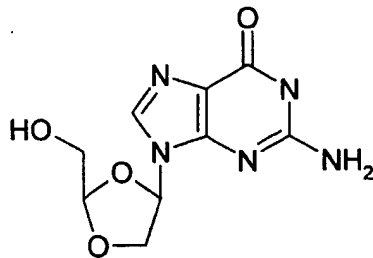


Compound 4 -cis-2-hydroxymethyl-4-(2'-6'-diamino-purin-9'-yl)-1,3-dioxolane; **Compound 4(-)** (-)-cis-2-hydroxymethyl-4-(2'-6'-diamino-purin-9'-yl)-1,3-dioxolane; **Compound 4(+)** (+)-cis-2-hydroxymethyl-4-(2'-6'-diamino-purin-9'-yl)-1,3-dioxolane

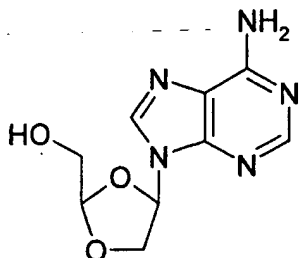


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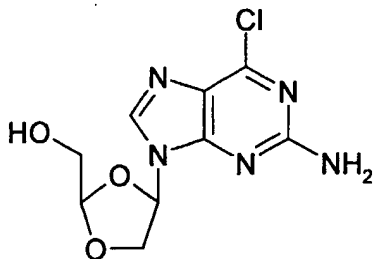
Compound 5 cis-2-hydroxymethyl-4-(guanin-9'-yl)-1,3-dioxolane; **Compound 5(-)** (-)-cis-2-hydroxymethyl-4-(guanin-9'-yl)-1,3-dioxolane; **Compound 5(+)** (+)-cis-2-hydroxymethyl-4-(guanin-9'-yl)-1,3-dioxolane



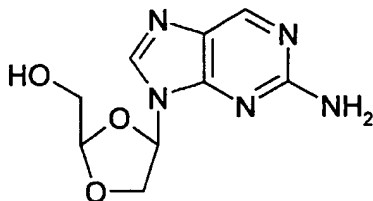
Compound 6 cis-2-hydroxymethyl-4-(adenin-9'-yl)-1,3-dioxolane; **Compound 6(-)** (-)-cis-2-hydroxymethyl-4-(adenin-9'-yl)-1,3-dioxolane; **Compound 6(+)** (+)-cis-2-hydroxymethyl-4-(adenin-9'-yl)-1,3-dioxolane



Compound 7 cis-2-hydroxymethyl-4-(2'-amino-6'-chloro-purin-9'-yl)-1,3-dioxolane; **Compound 7(-)** (-)-cis-2-hydroxymethyl-4-(2'-amino-6'-chloro-purin-9'-yl)-1,3-dioxolane; **Compound 7(+)** (+)-cis-2-hydroxymethyl-4-(2'-amino-6'-chloro-purin-9'-yl)-1,3-dioxolane

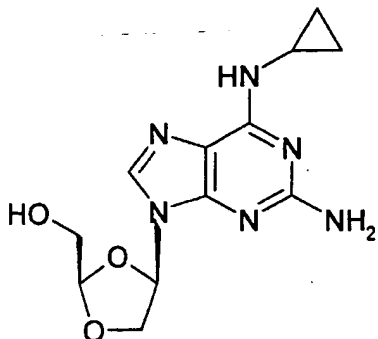


Compound 8 cis-2-hydroxymethyl-4-(2'-amino-purin-9'-yl)-1,3-dioxolane; **Compound 8(-)** (-)-cis-2-hydroxymethyl-4-(2'-amino-purin-9'-yl)-1,3-dioxolane; **Compound 8(+)** (+)-cis-2-hydroxymethyl-4-(2'-amino-purin-9'-yl)-1,3-dioxolane

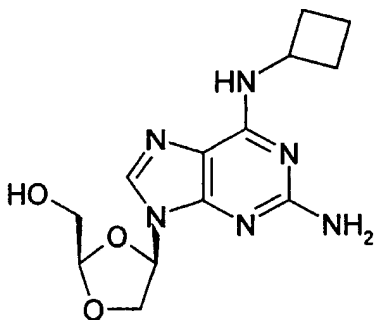


In a further aspect of the present invention compounds of
the invention include

Compound 1 (-) (-)-cis-2-hydroxymethyl-4-(2'-amino-6'-cyclopropylamino-purine-9'-yl)-1,3-dioxolane

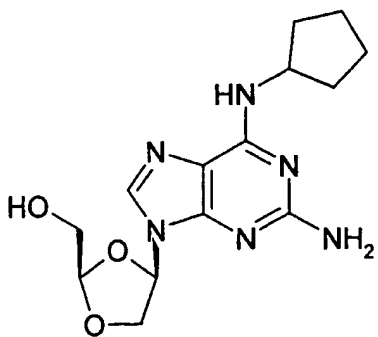


Compound 2 (-) (-)-cis-2-hydroxymethyl-4-(2'-amino-6'-cyclobutylamino-purine-9'-yl)-1,3-dioxolane

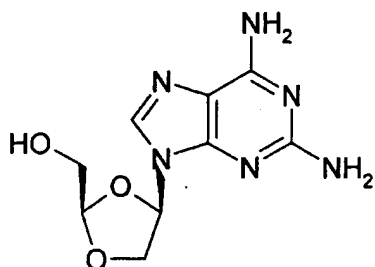


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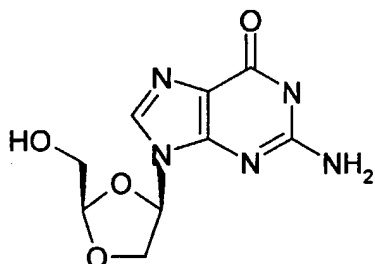
Compound 3 (-) (-)-cis-2-hydroxymethyl-4-(2'-amino-6'-cyclopentylamino-purine-9'-yl)-1,3-dioxolane



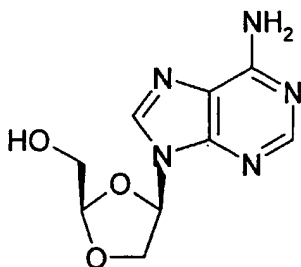
Compound 4 (-) (-)-cis-2-hydroxymethyl-4-(2'-6'-diamino-purin-9'-yl)-1,3-dioxolane



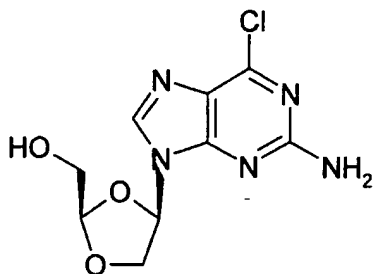
Compound 5 (-) (-)-cis-2-hydroxymethyl-4-(guanin-9'-yl)-1,3-dioxolane



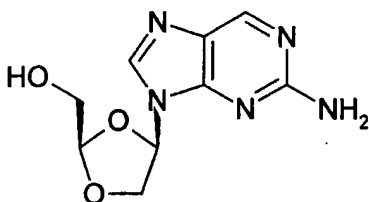
Compound 6 (-) (-)-cis-2-hydroxymethyl-4-(adenin-9'-yl)-1,3-dioxolane



Compound 7 (-) (-)-cis-2-hydroxymethyl-4-(2'-amino-6'-chloro-purin-9'-yl)-1,3-dioxolane

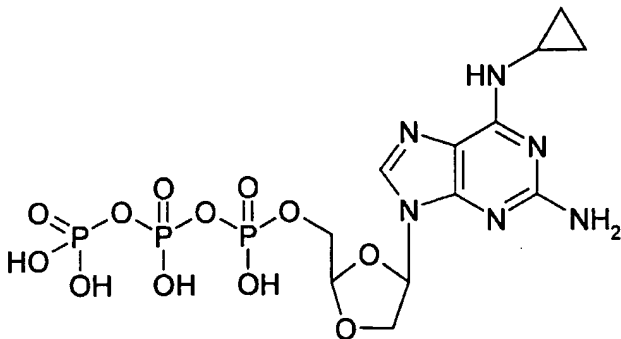


Compound 8(-) (-)-cis-2-hydroxymethyl-4-(2'-amino-purin-9'-yl)-1,3-dioxolane

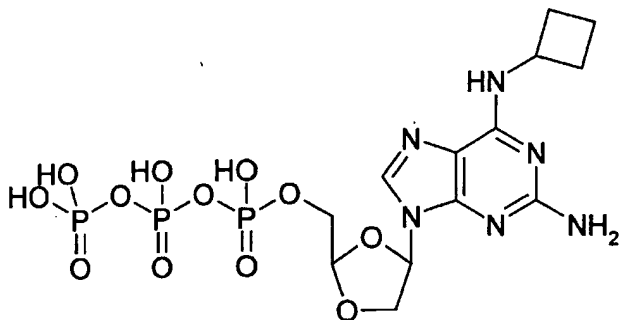


In a further embodiment, the compounds of the present invention have a triphosphate group in the 5' position. In a further aspect of the present invention, the compounds of
10 formula (I) are represented by:

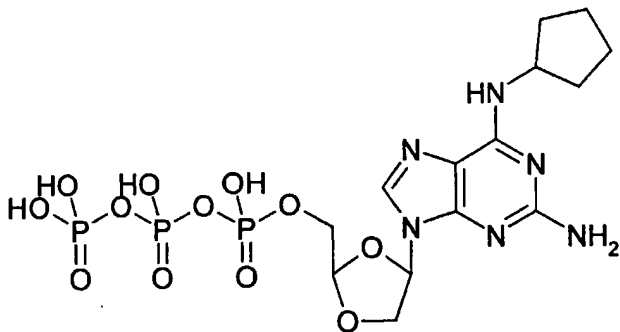
Compound 9 triphosphate-cis-2-hydroxymethyl-4-(2'-amino-6'-cyclopropylamino-purine-9'-yl)-1,3-dioxolane; **Compound 9(-)** (-)-triphosphate -cis-2-hydroxymethyl-4-(2'-amino-6'-cyclopropylamino-purine-9'-yl)-1,3-dioxolane; ; **Compound 9(+)** (+)-triphosphate -cis-2-hydroxymethyl-4-(2'-amino-6'-cyclopropylamino-purine-9'-yl)-1,3-dioxolane



Compound 10 Triphosphate -cis-2-hydroxymethyl-4-(2'-amino-6'-cyclobutylamino-purine-9'-yl)-1,3-dioxolane; **Compound 10(-)** (-)- triphosphate -cis-2-hydroxymethyl-4-(2'-amino-6'-cyclobutylamino-purine-9'-yl)-1,3-dioxolane **Compound 10(+)** (+)-triphosphate -cis-2-hydroxymethyl-4-(2'-amino-6'-cyclobutylamino-purine-9'-yl)-1,3-dioxolane

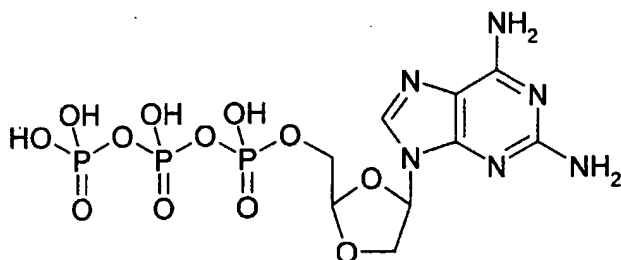


- 10 **Compound 11** Triphosphate cis-2-hydroxymethyl-4-(2'-amino-6'-cyclopentylamino-purine-9'-yl)-1,3-dioxolane; **Compound 11(-)** (-)- triphosphate -cis-2-hydroxymethyl-4-(2'-amino-6'-cyclopentylamino-purine-9'-yl)-1,3-dioxolane; **Compound 11(+)** (+)-triphosphate -cis-2-hydroxymethyl-4-(2'-amino-6'-cyclopentylamino-purine-9'-yl)-1,3-dioxolane

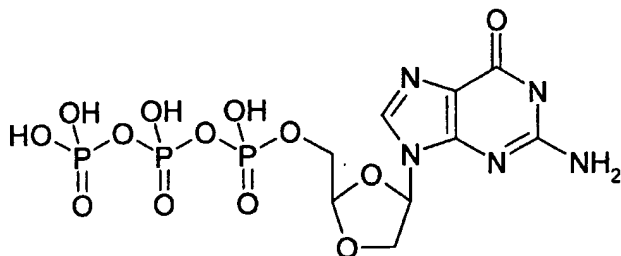


- Compound 12** Triphosphate cis-2-hydroxymethyl-4-(2'-6'-diamino-purin-9'-yl)-1,3-dioxolane; **Compound 12(-)** (-)- triphosphate -cis-2-hydroxymethyl-4-(2'-6'-diamino-purin-9'-yl)-1,3-dioxolane
- 20

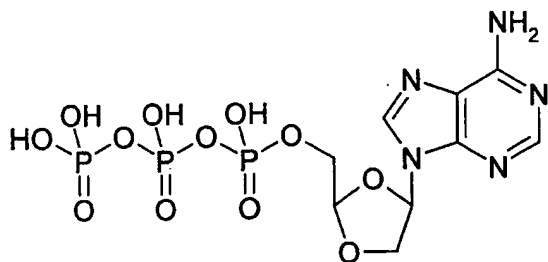
yl)-1,3-dioxolane; **Compound 12(+)** (+)-triphosphate -cis-2-hydroxymethyl-4-(2'-6'-diamino-purin-9'-yl)-1,3-dioxolane



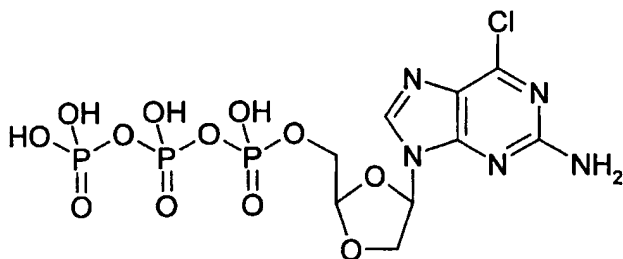
Compound 135 Triphosphate cis-2-hydroxymethyl-4-(guanin-9'-yl)-1,3-dioxolane; **Compound 135(-)** (-)-triphosphate -cis-2-hydroxymethyl-4-(guanin-9'-yl)-1,3-dioxolane; **Compound 13(+)** (+)-triphosphate -cis-2-hydroxymethyl-4-(guanin-9'-yl)-1,3-dioxolane



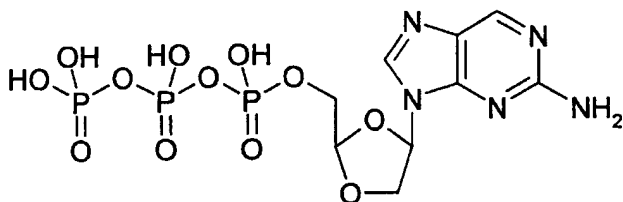
Compound 14 Triphosphate- cis-2-hydroxymethyl-4-(adenin-9'-yl)-1,3-dioxolane; **Compound 14(-)** (-)-triphosphate -cis-2-hydroxymethyl-4-(adenin-9'-yl)-1,3-dioxolane; **Compound 14(+)** (+)-triphosphate -cis-2-hydroxymethyl-4-(adenin-9'-yl)-1,3-dioxolane



Compound 15 Triphosphate -cis-2-hydroxymethyl-4-(2'-amino-6'-chloro-purin-9'-yl)-1,3-dioxolane; **Compound 15(-)** (-)-triphosphate -cis-2-hydroxymethyl-4-(2'-amino-6'-chloro-purin-9'-yl)-1,3-dioxolane; **Compound 15(+)** (+)-triphosphate -cis-2-hydroxymethyl-4-(2'-amino-6'-chloro-purin-9'-yl)-1,3-dioxolane

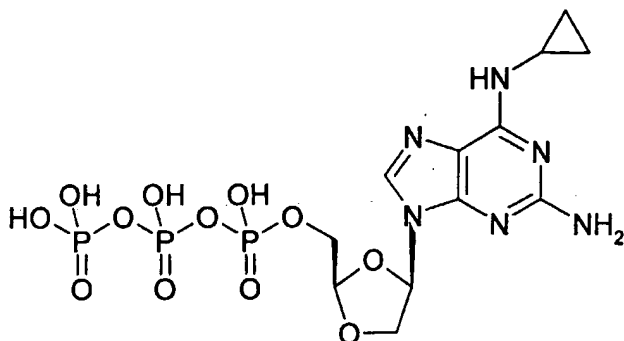


Compound 16 Triphosphate -cis-2-hydroxymethyl-4-(2'-amino-purin-9'-yl)-1,3-dioxolane; **Compound 16(-)** (-)-triphosphate -cis-2-hydroxymethyl-4-(2'-amino-purin-9'-yl)-1,3-dioxolane; **Compound 16(+)** (+)-triphosphate -cis-2-hydroxymethyl-4-(2'-amino-purin-9'-yl)-1,3-dioxolane

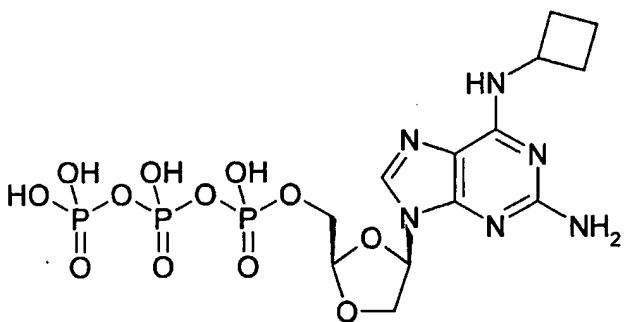


In a further aspect of the present invention compounds of the invention include:

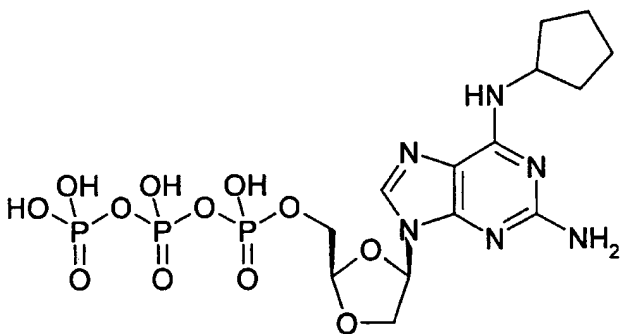
Compound 9(-) (-)-triphosphate-cis-2-hydroxymethyl-4-(2'-amino-6'-cyclopropylamino-purine-9'-yl)-1,3-dioxolane



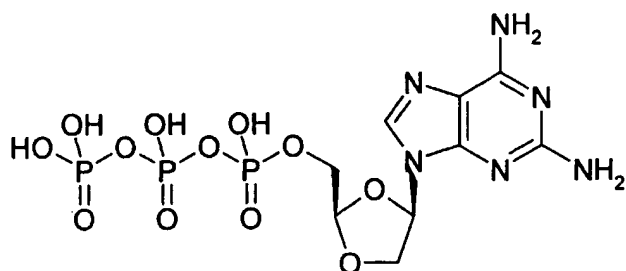
Compound 10(-) (-)- triphosphate -cis-2-hydroxymethyl-4-(2'-amino-6'-cyclobutylamino-purine-9'-yl)-1,3-dioxolane



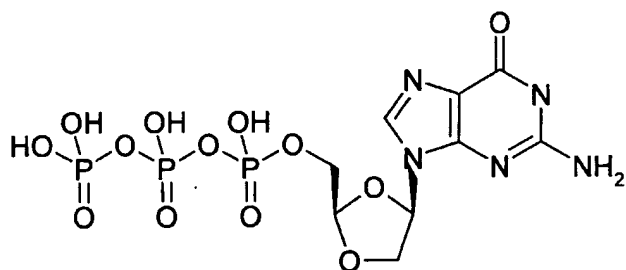
Compound 11(-) (-)- triphosphate -cis-2-hydroxymethyl-4-(2'-amino-6'-cyclopentylamino-purine-9'-yl)-1,3-dioxolane



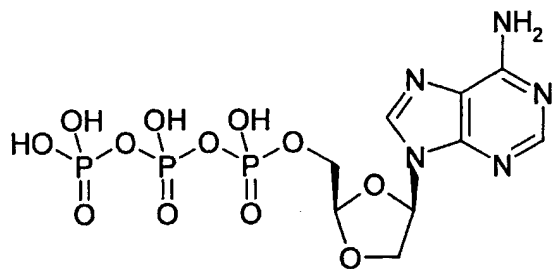
Compound 12(-) (-)-triphosphate -cis-2-hydroxymethyl-4-(2'-6'-diamino-purin-9'-yl)-1,3-dioxolane



Compound 13 (-) (-)-triphosphate -cis-2-hydroxymethyl-4-(guanine-9'-yl)-1,3-dioxolane

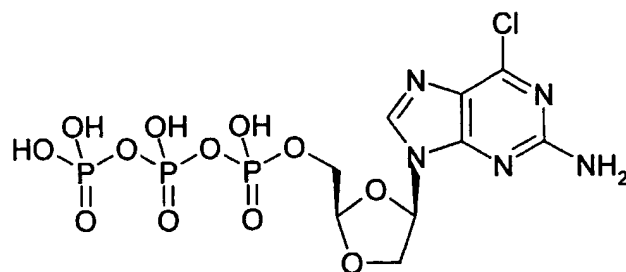


Compound 14 (-) (-)-triphosphate -cis-2-hydroxymethyl-4-(adenine-9'-yl)-1,3-dioxolane

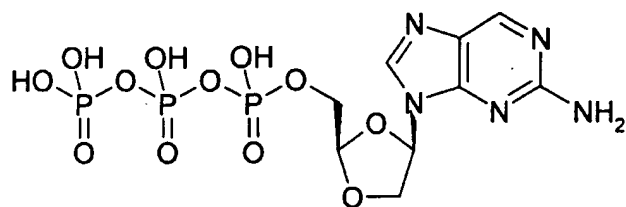


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Compound 15 (-) (-)-triphosphate -cis-2-hydroxymethyl-4-(2'-amino-6'-chloro-purin-9'-yl)-1,3-dioxolane

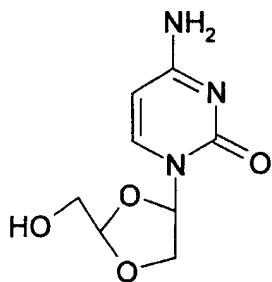


Compound 16 (-) (-)-triphosphate *cis*-2-hydroxymethyl-4-(2'-amino-purin-9'-yl)-1,3-dioxolane

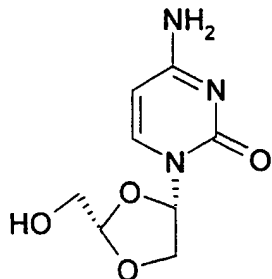


In a further embodiment of the present invention the compounds of formula (II) include:

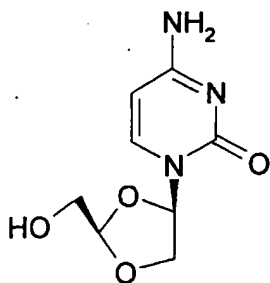
In one embodiment, a compound of formula II is *cis*-2-hydroxymethyl-4-(cytosin-1'-yl)-1,3-dioxolane (**compound#17**)



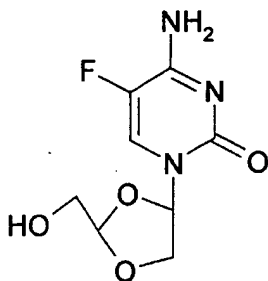
In one embodiment, a compound of formula II is (-)-*cis*-2-hydroxymethyl-4-(cytosin-1'-yl)-1,3-dioxolane (β -L-OddC) (**compound#17 (-)**) .



In one embodiment, a compound of formula II is (+)-*cis*-2-hydroxymethyl-4-(cytosin-1'-yl)-1,3-dioxolane (β -D-OddC) (**compound#17** (+)) .

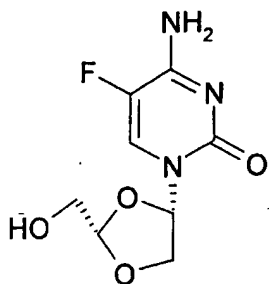


In another embodiment, a compound of formula II is *cis*-2-hydroxymethyl-4-(5'-fluorocytosin-1'-yl)-1,3-dioxolane (5FOddC) (**compound#18**) .

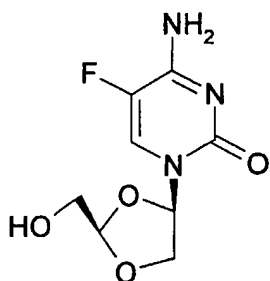


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In another embodiment, a compound of formula II is (-)-*cis*-2-hydroxymethyl-4-(5'-fluorocytosin-1'-yl)-1,3-dioxolane (β -L-5FOddC) (**compound#18** (-)) .

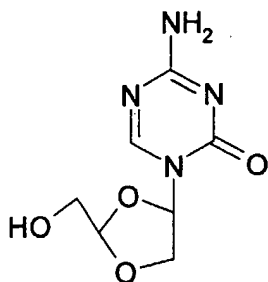


In another embodiment, a compound of formula II is (+)-*cis*-2-hydroxymethyl-4-(5'-fluorocytosin-1'-yl)-1,3-dioxolane(β -D-5F0ddC) (**compound#18 (+)**) .

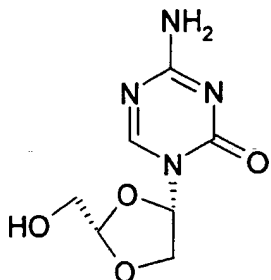


In another embodiment, a compound of formula II is *cis*-2-hydroxymethyl-4-(5'-azacytosin-1'-yl)-1,3-dioxolane (**compound#19**) .

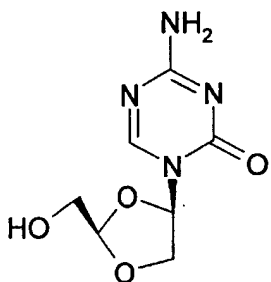
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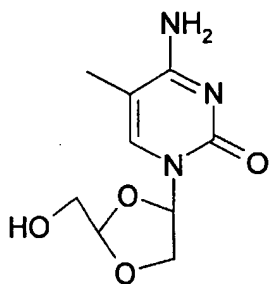
In another embodiment, a compound of formula II is (-)-*cis*-2-hydroxymethyl-4-(5'-azacytosin-1'-yl)-1,3-dioxolane (**compound#19 (-)**) .



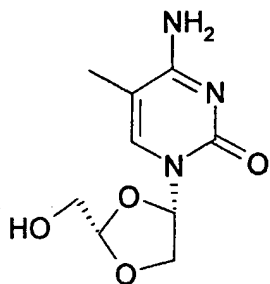
In another embodiment, a compound of formula II is (+)-*cis*-2-hydroxymethyl-4-(5'-azacytosin-1'-yl)-1,3-dioxolane (**compound#19** (+)) .



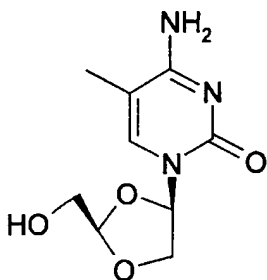
In another embodiment, a compound of formula II is *cis*-2-hydroxymethyl-4-(5'-methylcytosin-1'-yl)-1,3-dioxolane (**compound#20**) .



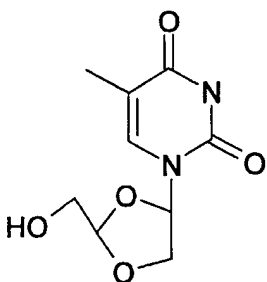
In another embodiment, a compound of formula II is (-)-*cis*-2-hydroxymethyl-4-(5'-methylcytosin-1'-yl)-1,3-dioxolane (**compound#20** (-)) .



In another embodiment, a compound of formula II is (+)-*cis*-2-hydroxymethyl-4-(5'-methylcytosin-1'-yl)-1,3-dioxolane (**compound#20** (+)) .

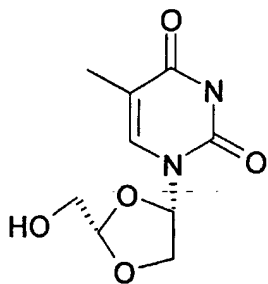


In another embodiment, a compound of formula II is *cis*-2-hydroxymethyl-4-(N-1'-thyminy)-1,3-dioxolane (**compound#21**) .

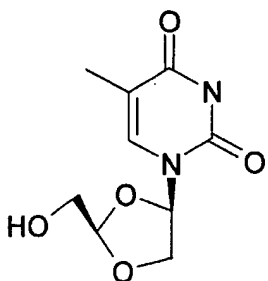


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In another embodiment, a compound of formula II is (-)-*cis*-2-hydroxymethyl-4-(N-1'-thyminy)-1,3-dioxolane (**compound#21** (-)) .

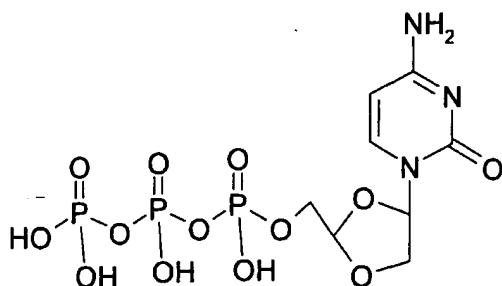


In another embodiment, a compound of formula II is (+)-*cis*-2-hydroxymethyl-4-(N-1'-thyminy)-1,3-dioxolane (compound#21 (+)) .

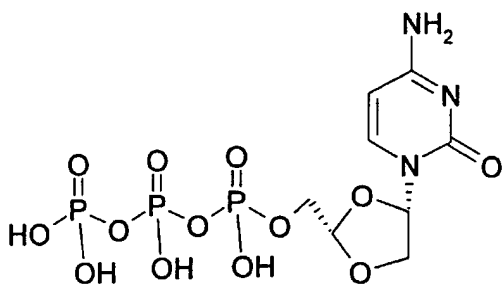


In a further embodiment, the compounds of the present invention have a triphosphate group in the 5' position. In a further aspect of the present invention, the compounds of formula (II) are represented by:

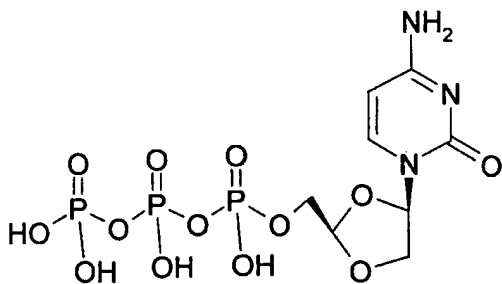
In one embodiment, a compound of formula II is triphosphate-*cis*-2-hydroxymethyl-4-(cytosin-1'-yl)-1,3-dioxolane (β -OddC-tp) (compound#22) .



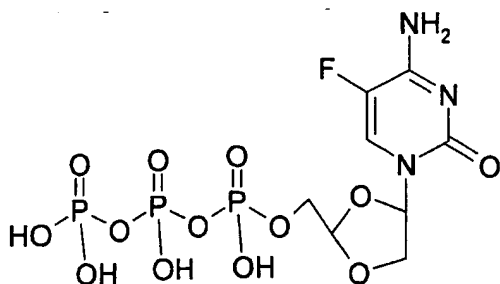
In one embodiment, a compound of formula II is (-)-triphosphate-*cis*-2-hydroxymethyl-4-(cytosin-1'-yl)-1,3-dioxolane (β -L-OddC-tp) (compound#22 (-)) .



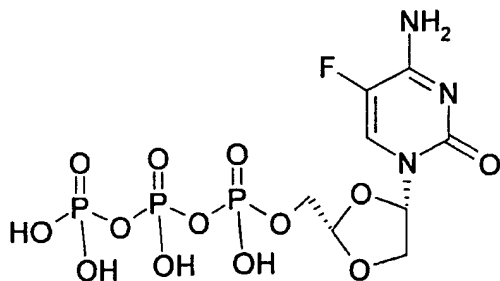
In one embodiment, a compound of formula II is (+)-triphosphate-*cis*-2-hydroxymethyl-4-(cytosin-1'-yl)-1,3-dioxolane (β -D-OddC-tp) (compound#22 (+)) .



In another embodiment, a compound of formula II is triphosphate-*cis*-2-hydroxymethyl-4-(5'-fluorocytosin-1'-yl)-1,3-dioxolane (β -5FOddC-tp) (compound#23).

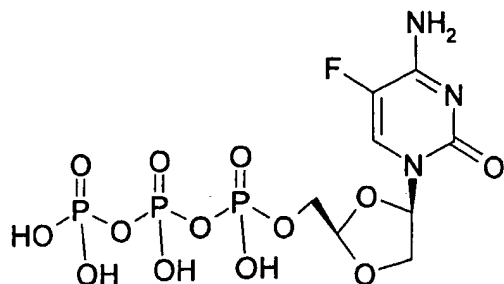


In another embodiment, a compound of formula II is (-)-triphosphate-*cis*-2-hydroxymethyl-4-(5'-fluorocytosin-1'-yl)-1,3-dioxolane (β -L-5FOddC-tp) (compound#23 (-)).

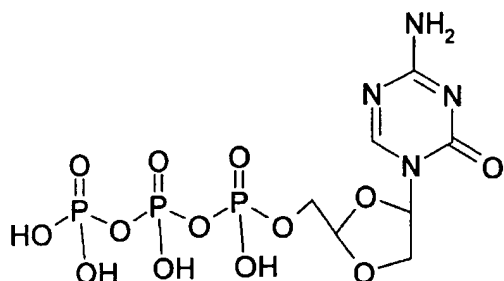


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In another embodiment, a compound of formula II is (+)-triphosphate-*cis*-2-hydroxymethyl-4-(5'-fluorocytosin-1'-yl)-1,3-dioxolane (β -D-5FOddC-tp) (compound#23 (+)).

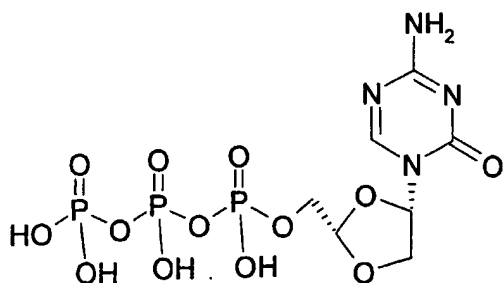


In another embodiment, a compound of formula II is triphosphate-*cis*-2-hydroxymethyl-4-(5'-azacytosin-1'-yl)-1,3-dioxolane (compound#24) .

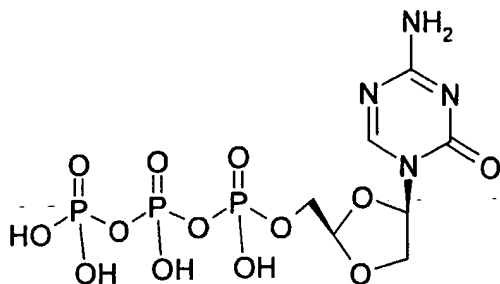


In another embodiment, a compound of formula II is (-)-triphosphate-*cis*-2-hydroxymethyl-4-(5'-azacytosin-1'-yl)-1,3-dioxolane (compound#24 (-)) .

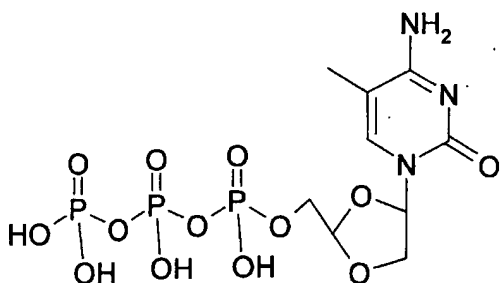
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In another embodiment, a compound of formula II is (+)-triphosphate-*cis*-2-hydroxymethyl-4-(5'-azacytosin-1'-yl)-1,3-dioxolane (compound#24 (+)) .

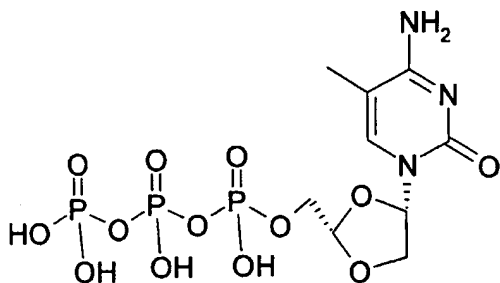


In another embodiment, a compound of formula II is triphosphate-*cis*-2-hydroxymethyl-4-(5'-methylcytosin-1'-yl)-1,3-dioxolane (compound#25).

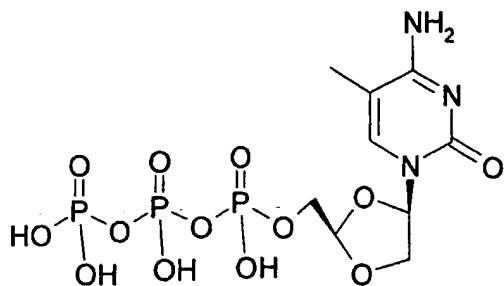


In another embodiment, a compound of formula II is (-)-triphosphate-*cis*-2-hydroxymethyl-4-(5'-methylcytosin-1'-yl)-1,3-dioxolane (compound#25 (-)).

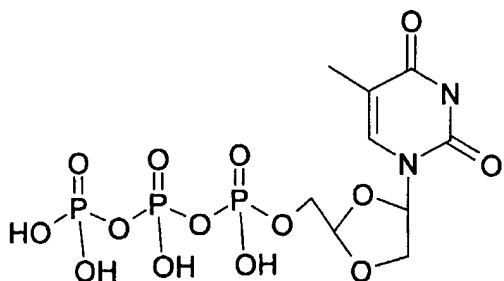
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In another embodiment, a compound of formula II is (+)-triphosphate-*cis*-2-hydroxymethyl-4-(5'-methylcytosin-1'-yl)-1,3-dioxolane (compound#25 (+)).

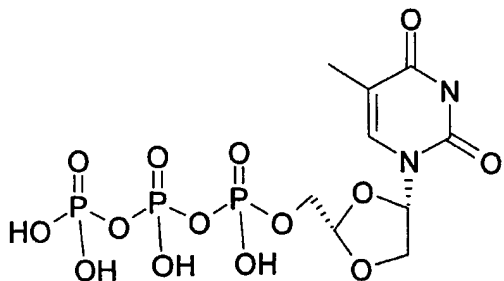


In another embodiment, a compound of formula II is triphosphate-*cis*-2-hydroxymethyl-4-(N-1'-thyminy)-1,3-dioxolane (compound#26).

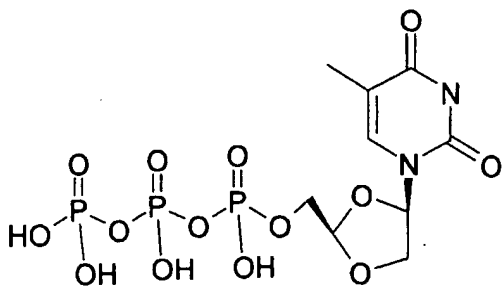


In another embodiment, a compound of formula II is (-)-triphosphate-*cis*-2-hydroxymethyl-4-(N-1'-thyminy)-1,3-dioxolane (compound#26 (-)).

10



In another embodiment, a compound of formula II is (+)-triphosphate-*cis*-2-hydroxymethyl-4-(N-1'-thyminy)-1,3-dioxolane (compound#26 (+)).



In one embodiment the compounds of the present invention are provided in the form of a single enantiomer at least 95% free of the corresponding enantiomer:

In one embodiment the compounds of the present invention are provided in the form of a single enantiomer at least 95% free of the corresponding enantiomer.

- 10 In one embodiment the compounds of the present invention are provided in the form of a single enantiomer at least 99% free of the corresponding enantiomer.

In an other embodiment the compound of the present invention are in the form of the (+) enantiomer at least 95% free of the corresponding (-) enantiomer.

In an other embodiment the compound of the present invention are in the form of the (+) enantiomer at least 97% free of the corresponding (-) enantiomer.

- 20 In an other embodiment the compound of the present invention are in the form of the (+) enantiomer at least 99% free of the corresponding (-) enantiomer.

In an other embodiment, the compound of the present invention are in the form of the (-) enantiomer at least 95% free of the corresponding (+) enantiomer.

In an other embodiment the compound of the present invention are in the form of the (-) enantiomer at least 97% free of the corresponding (+) enantiomer.

In an other embodiment the compound of the present invention are in the form of the (-) enantiomer at least 99% free of the corresponding (+) enantiomer.

- There is also provided a pharmaceutically acceptable salts of the present invention. By the term pharmaceutically acceptable salts of compounds of general formula (I) and (II) are meant those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples
- 10 of suitable acids include hydrochloric, hydrobromic, sulphuric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, lactic, salicylic, succinic, toleune-p-sulphonic, tartaric, acetic, citric, methanesulphonic, formic, benzoic, malonic, naphthalene-2-sulphonic and benzenesulphonic acids. Other acids such as oxalic, while not in themselves pharmaceutically acceptable, may be useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts.
- 20 Salts derived from appropriate bases include alkali metal (e.g. sodium), alkaline earth metal (e.g. magnesium), ammonium and NR_4^+ (where R is C_{1-4} alkyl) salts.

References hereinafter to a compound according to the invention includes compounds of the general formula (I) and (II) and there pharmaceutically acceptable salts.

- Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by
- 30 one of ordinary skill in the art to which this invention belongs. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

As used in this application, the term "alkyl" represents an unsubstituted or substituted (by a halogen, nitro, CONH_2 , COOH , O-C_{1-6} alkyl, O-C_{2-6} alkenyl, O-C_{2-6} alkynyl, hydroxyl, amino, or COOQ , wherein Q is C_{1-6} alkyl; C_{2-6} alkenyl; C_{2-6} alkynyl) straight chain, branched chain or cyclic hydrocarbon moiety (e.g. isopropyl, ethyl, fluorohexyl or cyclopropyl). The term alkyl is also meant to include alkyls in which one or more hydrogen atoms is replaced by an
10 halogen, more preferably, the halogen is fluoro (e.g. CF_3 - or CF_3CH_2 -).

The terms "alkenyl" and "alkynyl" represent an alkyl containing at least one unsaturated group (e.g. allyl).

The term "cycloalkyl" represents an alkyl which is cyclic, such as cyclopropyl, cyclopentyl or cyclobutyl.

The term "hydroxy protecting group" is well known in the
20 field of organic chemistry. Such protecting groups may be found in T. Greene, Protective Groups In Organic Synthesis, (John Wiley & Sons, 1981). Example of hydroxy protecting groups include but are not limited to acetyl-2-thioethyl ester, pivaloyloxymethyl ester and isopropylloxycarbonyloxymethyl ester.

The term "aryl" represent an unsaturated carbocyclic moiety, optionally mono- or di-substituted with OH, SH, amino, halogen or C_{1-6} alkyl.

30

The term "heteroaryl" represent an aryl wherein at least one carbon ring atom is substituted by a heteroatom (e.g. N, O, or S).

The term "aminoalkyl" represent an alkyl which is covalently bonded to the adjacent atom through a nitrogen atom.

The term "thioalkyl" represent an alkyl which is covalently bonded to the adjacent atom through a sulfur atom.

The term "alkoxy" represent an alkyl which is covalently bonded to the adjacent atom through an oxygen atom.

When there is a sulfur atom present, the sulfur atom can be at different oxydation level, S, SO, or SO₂. All such
10 oxydation level are within the scope of the present invention.

The term "host" represent any mammals including humans.

In one embodiment, the host is human.

The compounds of the present invention can be prepared by methods well known in the art. For example, such methods are described in the following references: US patent 5,041,449,
20 PCT publication WO 92/20669 (PCT application PCT/CA92/00211), Journal of Chromatography, 645 (1993) 107-114, Tetrahedron Assymetry Vol. 4 No. 11 pp2319-2322 (1993), Tetrahedron Assymetry Vol. 4 No. 2 pp211-214 (1993), Bioorganic & Medicinal Chemistry Vol.3 No.8, pp.1543-1546 (1993), Tetrahedron Letters, Vol.33, No. 46, pp 6949-6952, (1992), J.Org. Chem., 34(6), 1547-1550 (1969), J.Org. Chem., 52(9), 1794-1801 (1987), J.Am.Chem.Soc., 87(8), 1785-1788 (1965), J.Org. Chem. (1989), 54, 631-635. which are all incorporated by reference.

30

According to one embodiment, it will be appreciated that the amount of a compound of the present invention required for use in treatment will vary not only with the particular compound selected but also with the route of administration, the nature of the condition for which treatment is required and the age and condition of the patient and will be

ultimately at the discretion of the attendant physician or veterinarian. In general however a suitable dose will be in the range of from about 0.01 to about 750 mg/kg of body weight per day, preferably in the range of 0.5 to 60 mg/kg/day, most preferably in the range of 1 to 20 mg/kg/day.

The desired dose according to one embodiment is conveniently presented in a single dose or as divided dose administered at appropriate intervals, for example as two, three, four or more doses per day.

In another embodiment, the compound is conveniently administered in unit dosage form; for example containing 10 to 1500 mg, conveniently 20 to 1000 mg, most conveniently 50 to 700 mg of active ingredient per unit dosage form.

According to another embodiment of the present invention, the active ingredient is administered to achieve peak plasma concentrations of the active compound of from about 1 to about 75 μ M, preferably about 2 to 50 μ M, most preferably about 3 to about 30 μ M. This may be achieved, for example, by the intravenous injection of a 0.1 to 5% solution of the active ingredient, optionally in saline, or orally administered as a bolus containing about 1 to about 500 mg of the active ingredient. Desirable blood levels may be maintained by a continuous infusion to provide about 0.01 to about 5.0 mg/kg/hour or by intermittent infusions containing about 0.4 to about 15 mg/kg of the active ingredient.

While it is possible that, for use in therapy, a compound of the present invention may be administered as the raw chemical, it is preferable according to one embodiment of the invention, to present the active ingredient as a pharmaceutical formulation. The embodiment of the invention thus further provides a pharmaceutical formulation

comprising a compound of formula (I), or formula (II), or a pharmaceutically acceptable salt thereof together with one or more pharmaceutically acceptable carriers therefor and, optionally, other therapeutic and/or prophylactic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

10 According to one embodiment of the present invention, pharmaceutical formulations include but are not limited to those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), transdermal, vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration or in a form suitable for administration by inhalation or insufflation. The formulations may, where appropriate, be conveniently presented in discrete dosage units and may be prepared by any of the methods well known in the art of pharmacy. All methods according to this
20 embodiment include the step of bringing into association the active compound with liquid carriers or finely divided solid carriers or both and then, if necessary, shaping the product into the desired formulation.

According to another embodiment, pharmaceutical formulation suitable for oral administration are conveniently presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules. In another embodiment, the formulation is presented as a solution, a suspension or as
30 an emulsion. Still in another embodiment, the active ingredient is presented as a bolus, electuary or paste. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrants, or wetting agents. The tablets may be coated according to methods well known in the art. Oral liquid preparations may be in the form of, for example,

aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives.

10 The compounds of the present invention according to an embodiment are formulated for parenteral administration (e.g. by injection, for example bolus injection or continuous infusion) and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile
20 solid or by lyophilisation from solution, for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use.

For topical administration to the epidermis, the compounds, according to one embodiment of the present invention, are formulated as ointments, creams or lotions, or as a transdermal patch. Such transdermal patches may contain penetration enhancers such as linalool, carvacrol, thymol, citral, menthol and t-anethole. Ointments and creams may,
30 for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents, thickening agents, or colouring agents.

Formulations suitable for topical administration in the mouth include lozenges comprising active ingredient in a flavoured base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

- Pharmaceutical formulations suitable for rectal
- 10 administration wherein the carrier is a solid. In another embodiment, they are presented as unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art, and the suppositories may be conveniently formed by admixture of the active compound with the softened or melted carrier(s) followed by chilling and shaping in moulds.

- According to one embodiment, the formulations suitable for vaginal administration are presented as pessaries, tampons,
- 20 creams, gels, pastes, foams or sprays containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

- For intra-nasal administration the compounds, in one embodiment of the invention, are used as a liquid spray or dispersible powder or in the form of drops. Drops may be formulated with an aqueous or non-aqueous base also comprising one more dispersing agents, solubilising agents or suspending agents. Liquid sprays are conveniently
- 30 delivered from pressurized packs.

For administration by inhalation the compounds, according to one embodiment of the invention are conveniently delivered from an insufflator, nebulizer or a pressurized pack or other convenient means of delivering an aerosol spray. In another embodiment, pressurized packs comprise a suitable

propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In another embodiment, the dosage unit in the pressurized aerosol is determined by providing a valve to deliver a metered amount.

- Alternatively, in another embodiment, for administration by inhalation or insufflation, the compounds according to the present invention are in the form of a dry powder
- 10 composition, for example a powder mix of the compound and a suitable powder base such as lactose or starch. In another embodiment, the powder composition is presented in unit dosage form in, for example, capsules or cartridges or e.g. gelatin or blister packs from which the powder may be administered with the aid of an inhalator or insufflator.

In one embodiment, the above described formulations are adapted to give sustained release of the active ingredient.

- 20 The compounds of the invention may also be used in combination with other antiviral agents.

In one embodiment, the compounds of the invention may be employed together with at least one other antiviral agent chosen from protease inhibitors, polymerase inhibitors, and helicase inhibitors.

- As used in this application, the term "interferon" include: interferon like molecules such as interferon (IFN),
- 30 interferon α -2a, interferon α -2b, consensus interferon (CIFN) and other types of interferons.

In one embodiment, the compounds of the invention may be employed together with at least one other antiviral agent chosen from interferon (IFN), interferon α -2a, interferon α -

2b, consensus interferon (CIFN), ribavirin, amantadine, rimantadine, interleukine-12, ursodeoxycholic acid (UDCA), glycyrrhizin and silybum marianum.

In one embodiment, the compounds of the invention may be employed together with at least one other antiviral agent chosen from Interferon- α , Ribavirin and Amantadine.

10 In one embodiment, the compounds of the invention may be employed together with at least one other antiviral agent chosen from Interferon- α and Ribavirin (REBETRON).

In one embodiment, the compounds of the invention may be employed together Interferon- α .

In one embodiment, the compounds of the invention may be employed together with Ribavirin.

20 The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus pharmaceutical formulations comprising a combination as defined above together with a pharmaceutically acceptable carrier therefor comprise a further aspect of the invention.

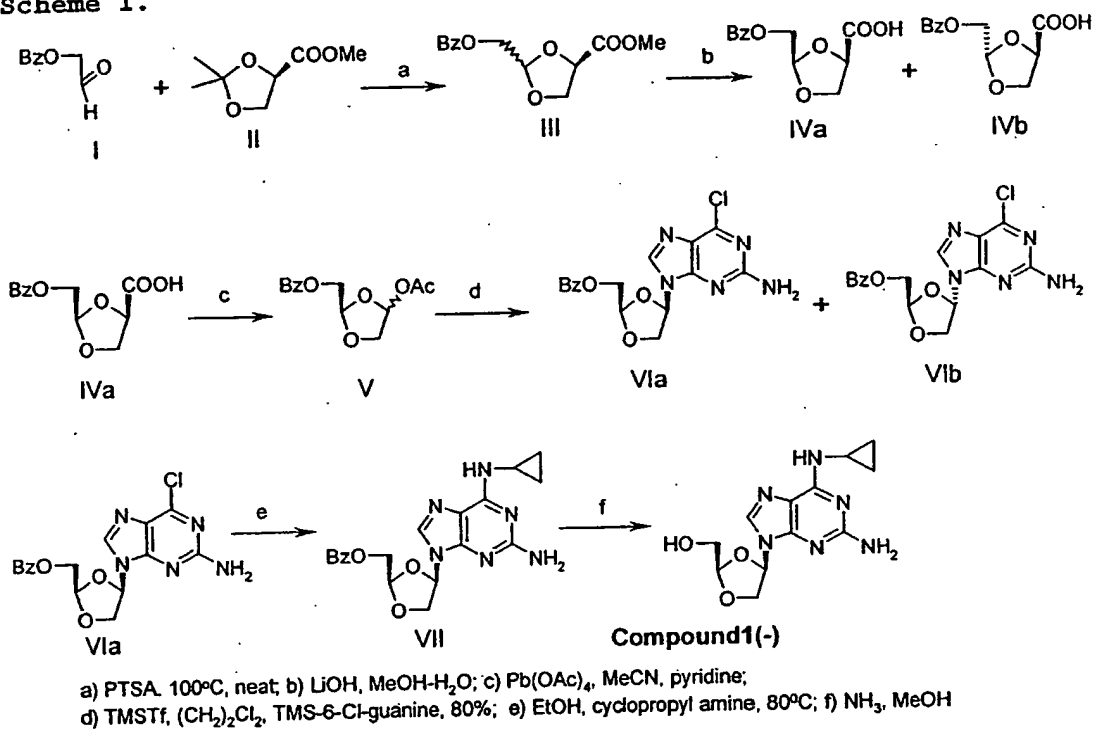
The individual components of such combinations may be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations.

30 When the compound (I), or (II) or a pharmaceutically acceptable salts thereof is used in combination with a second therapeutic agent active against the same virus the dose of each compound may be either the same as or differ from that when the compound is used alone. Appropriate

doses will be readily appreciated by those skilled in the art.

The following examples are provided to illustrate various embodiments of the present invention and shall not be considered as limiting in scope.

Scheme 1.



10

The target compound can be prepared according to the above scheme :

Step a : 2-benzoyloxy-acetaldehyde I reacted with methyl (R) - (+) - 2,2-dimethyl-1,3-dioxolane-4-carboxylate II in the presence para-toluene sulfonic acid (pTSA) under transketalisation to give 2-benzoyloxymethyl-1,3-dioxolane-4-carboxylmethyl ester III as a mixture of cis and trans isomers in a ratio of 3 :1 in favor of cis isomer.

Step b : The carboxylic methyl ester III was selectively hydrolysed using lithium hydroxide to give the corresponding acid derivatives IVa and IVb. The mixture were separated by flash chromatography and each isomer was further used independently..

Step c : The carboxylic function of IVa was then converted to an acetoxy leaving group by treatment with lead tetraacetate.

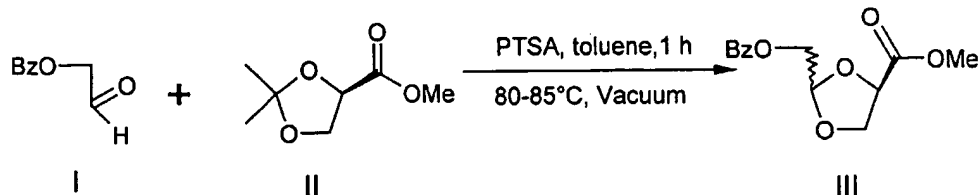
Step d : The (2R)-2-benzoyloxymethyl-1,3-dioxolane-4-acetoxy IVa was coupled with silylated 2-amino-6-chloropurine using trimethylsilyl trifluoromethylsulfonate (TMSTf) as activator to give a mixture of cis and trans isomers of nucleoside analogues VIa and VIb in a ratio of 1.2 :1 in favor of cis isomer. The mixture was separated by flash chromatography and each isomer was used independently further.

Step e : The (-)-(2R, 4R)-2-benzoyloxymethyl-4-(2'-amino-6'-chloro-purine-9'-yl)-1,3-dioxolane VIa was treated with cyclopropylamine in ethanol to give the corresponding (-)-(2R,4R)-2-benzoyloxymethyl-4-(2'-amino-6'-cyclopropylamino-purine-9'-yl)-1,3-dioxolane VII in good yield.

Step f : Removal of benzoyl protecting group was achieved by treatment of (-)-(2R,4R)-2-benzoyloxymethyl-4-(2'-amino-6'-cyclopropylamino-purine-9'-yl)-1,3-dioxolane VII with methanolic ammonia to give the desired product (-)-(2R,4R)-2-hydroxymethyl-4-(2'-amino-6'-cyclopropylamino-purine-9'-yl)-1,3-dioxolane 1(-) in good yield.

Example 1.

Methyl-2-(R,S)-benzoyloxymethyl-1,3-dioxolane-4-(R)-carboxylate. (III)



To a solution of methyl- α,β -isopropylidene-D-glycerate (II) (Fluka: registry # 52373-72-5), (9.76g, 60.9 mmol, 1 eq) and benzoyloxyacetaldehyde I (10g, 60.9 mmol, 1 eq) in toluene (20 mL) at 80°C, p-toluenesulfonic acid (PTSA) (460 mg, 2.4 mmol, 4 mol %) was added. The reaction flask was kept under vacuum for one hour and a distillate was collected (80-85°C) during this period of time. The residue was then cooled to room temperature (RT) and purified by column chromatography on silica gel, using hexanes/ethyl acetate as eluent to produce 13.2 g (81%) of the title compound as a mixture of cis and trans isomers in a ratio of 3:1.

Cis isomer:

$^1\text{H-NMR}$ (CDCl_3) : δ (ppm) : 3.75 (s, 3H, CH_3); 4.15 (dd, 1H, $\text{C}_5\text{-CH}$), 4.30 (dd, 1H, $\text{C}_5\text{-CH}$); 4.5 (m, 2H, $\text{CH}_2\text{-O-CO-C}_6\text{H}_5$); 4.7 (m, 1H, $\text{C}_4\text{-CH}$); 5.4 (t, 1H, $\text{C}_2\text{-CH}$); 7.45-8.1 (m, 5H, Ar-CH).

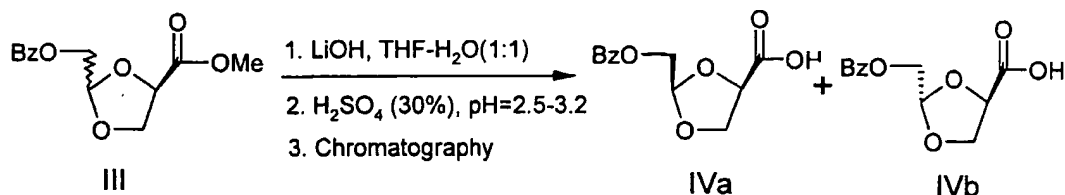
Trans isomer:

$^1\text{H-NMR}$ (CDCl_3) : δ (ppm) : 3.8 (s, 3H, CH_3); 4.1 (dd, 1H, $\text{C}_5\text{-CH}$); 4.35 (dd, 1H, $\text{C}_5\text{-CH}$); 4.45 (m, 2H, $\text{CH}_2\text{-O-CO-C}_6\text{H}_5$); 4.75 (m, 1H, $\text{C}_4\text{-CH}$); 5.5 (t, 1H, $\text{C}_2\text{-CH}$); 7.45-8.1 (m, 5H, Ar-CH).

Example 2.

(2R, 4R)-2-benzoyloxymethyl-1,3-dioxolane-4-carboxylic acid. (IVa)

(2S, 4R)-2-benzoyloxymethyl-1,3-dioxolane-4-carboxylic acid. (IVb)



To a solution of methyl-2-(R,S)-benzoyloxymethyl-1,3-dioxolane-4-(R)-carboxylate (III), (411 g, 1.54 mmol, 1 eq., 2 :1 mixture of cis and trans isomers) in a 1:1 mixture of tetrahydrofuran (THF) and water, lithium hydroxide (LiOH) (64.8 g, 1.54 moles, 1 eq) was added portion wise over a period of 30 min., keeping the reaction flask temperature below 30°C. After 90 min., THF was removed by vacuum and the aqueous solution was acidified to pH= 2.5-3.2, by dropwise addition of 30% (w/w) sulphuric acid. The

10 resulting solution was extracted with dichloromethane (4X400 mL). The combined organic phase was washed with brine, dried over sodium sulphate and concentrated to produce 380g of a dark oil. The isomers were separated by column chromatography on silica gel, using 2% acetic acid in dichloromethane to produce 220g of the cis isomer (IVa) (56.5%) and 116g of the trans isomer (IVb) (30%). Each of isomers was independently used for next step.

Cis isomer:

(2R, 4R)-2-benzoyloxymethyl-1,3-dioxolane-4-carboxylic
20 acid.(IVa)

¹H-NMR (CDCl₃) : δ(ppm) : 4.2 (t, 1H, C₅-H); 4.4 (m, 1H); 4.5 (m, 1H); 4.7 (m, 2H); 5.4 (t, 1H, C₂-CH); 7.45-8.1 (m, 5H, Ar-CH); 7.2-8.0(bs, 1H, COOH).

Trans isomer:

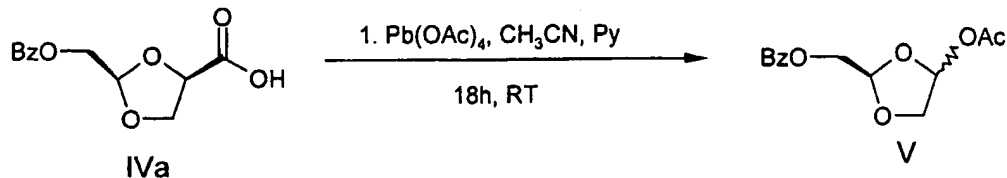
(2S, 4R)-2-benzoyloxymethyl-1,3-dioxolane-4-carboxylic
acid.(IVb)

¹H-NMR (CDCl₃) : δ(ppm) : 4.15 (dd, 1H, C₅-H); 4.4 (t, 1H, C₅-H); 4.45 (m, 2H, CH₂-OCOC₆H₅); 4.8 (dd, 1H, C₄-CH); 5.6 (t, 1H, C₂-CH); 7.45-8.1 (m, 5H, Ar-CH); 8.3-8.8 (bs, 1H, COOH).

30

Example 3.

(2R)-2-benzoyloxymethyl-4-(R,S)-acetoxy-1,3-dioxolane.(V)



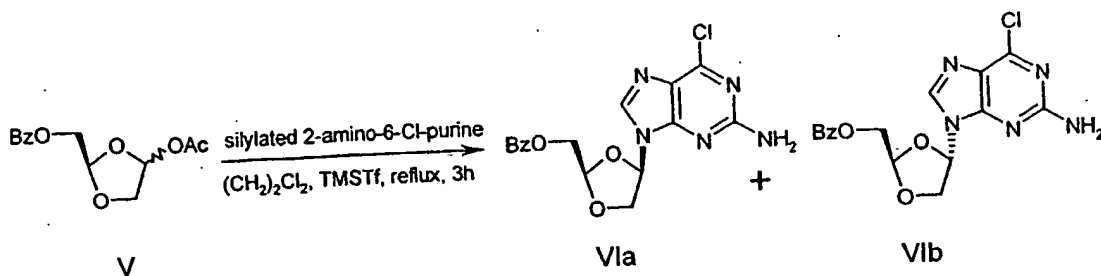
To a solution of (2R,4R)-2-benzoyloxymethyl-1,3-dioxolane-4-carboxylic acid (IVa), (130g, 0.515moles, 1eq) and pyridine (60 mL, 0.741moles, 1.44eq) in acetonitrile at 4°C, lead tetraacetate. (assay 95%, 300g, 0.678 moles, 1.25 eq) was added over a period of 20 min. The reaction mixture was kept under stirring for 18 hours at room temperature (RT). The inorganics were removed by filtration, the filtrate was poured on to a saturated solution of sodium bicarbonate (2L) followed by addition of solid sodium bicarbonate (pH = 7-8). The organic phase was separated, and the aqueous phase was extracted with ethyl acetate (3X400 mL). The combined organic phase was concentrated and purified by column chromatography, on silica gel, using hexanes/ethyl acetate as eluent to produce 93.5g (68%) of the title compound as a mixture of cis and trans isomers in a ratio of 2:1 (V). The mixture was used for next step.

20 cis/trans isomers:

$^1\text{H-NMR}$ (CDCl_3) : δ (ppm): 2.0, 2.15 (s, 3H, CH_3); 4.05-4.45 (m, 4H, CH); 5.45, 5.55 (t, 1H, $\text{C}_2\text{-CH}$); 6.4, 6.45 (dd, 1H, $\text{C}_4\text{-CH}$); 7.45-8.1 (m, 5H, Ar- CH);

Example 4.

(2R,4R) and (2R,4S)-2-benzoyloxymethyl-4-(2'-amino-6'-chloro-purine-9'-yl)-1,3-dioxolane (VIa and VIb)



2-amino-6-chloro-purine (4.15 g, 1.3eq.) in 50ml of hexamethyldisilazane(HMDS) containing 100 mg of ammonium sulfate was heated under reflux for 3h after which time the clear solution was evaporated to dryness in vacuo. The residue was dissolved in 100mL of anhydrous 1,2-dichloroethane. (2R)-2-benzoyloxymethyl-4-acetoxy-1,3-dioxolane (V) (5g) was dried by co-evaporation twice with benzene (2x30mL) and dissolved in 100 mL of anhydrous 1,2-dichloroethane. The solution was then transferred into the reaction flask containing silylated 2-amino-6-chloro-purine solution. The mixture was placed in a 60°C preheated oil bath for 15 minutes, followed the addition of trimethylsilyl triflate (TMS-OTf) (3.8 mL, 1.1eq.). The mixture was heated at refluxing under nitrogen for 3h and the solution became brown. TLC (hex :EtOAc 7 :3 for sugar and hex :EtOAc 1 :4 for product) indicated a completed reaction with the disappearance of sugar and the presence of two well separated spots for cis and trans products. The reaction mixture was cooled to room temperature, poured into a saturated sodium bicarbonate solution (100 mL) and stirred for 10 minutes. The organic layer was collected and the aqueous layer was extracted twice with methylene chloride (2 x 50 ml). The combined organic solution was washed with water, brine and dried over MgSO₄ as usual. and solvent was evaporated to dryness to give a foam (7g). H-NMR of the crude indicated clean reaction with cis and trans products in a ratio of 1.2 :1 in favor of cis isomer. The crude

product was purified on silica gel using a gradient of hexane : ethyl acetate 7 : 3, 1 : 1 and 2 : 3 as eluant to yield 2.5 g of trans isomer (less polar, α -anomer) (VIb) as a foam, which was crystallized in EtOH and 3g of cis isomer (more polar, β -anomer) (VIa) as a foam, which was crystallized in EtOH and 0.3g of mixture cis and trans in favor of cis as a foam for a total of 82% yield.

Trans isomer :

- 10 (+) - (2R,4S) - 2-benzoyloxymethyl-4-(2'-amino-6'-chloro-purine-9'-yl)-1,3-dioxolane (VIb)

R_f : 0.40 (hexane-EtOAc 3 : 7)

[α _D] +21.16° (c 0.293 in CH₂Cl₂)

¹H-NMR (CDCl₃) : δ (ppm) : 4.45-4.55 (m, 4H ; C₅-H₂, C₂-CH₂-OBz), 5.16 (b, 2H, NH₂), 5.83 (t, 1H, C₂-H, J=3.8Hz), 6.39 (dd, 1H, C₄-H), 7.45 (t, 2H, aromatic), 7.62 (t, 1H, aromatic), 7.92 (s, 1H, C₈-H), 8.10 (d, 2H, aromatic).

U.V. : (CH₃OH) λ_{\max} : 312nm

- 20 Cis isomer :

(-) - (2R,4R) - 2-benzoyloxymethyl-4-(2'-amino-6'-chloro-purine-9'-yl)-1,3-dioxolane (VIa)

R_f : 0.26 (hexane-EtOAc 3 : 7)

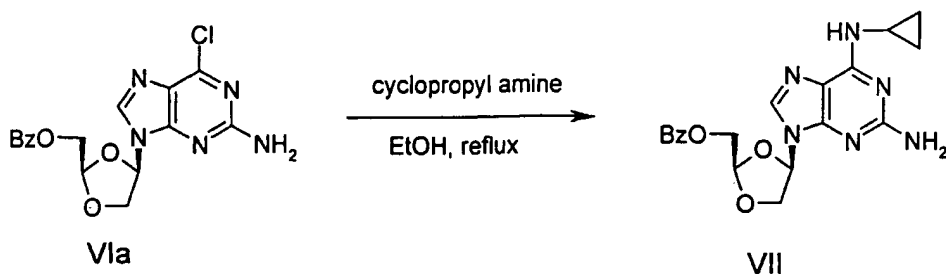
[α _D] -87.7° (c 0.2565 in CH₂Cl₂)

¹H-NMR (CDCl₃) : δ (ppm) : 4.25-4.33 (dd, 1H, C₅-H), 4.60-4.64 (m, 3H ; C₅-H and C₂-CH₂-OBz), 5.17 (b, 2H, NH₂), 5.42 (t, 1H, C₂-H, J=3.5Hz), 6.33 (dd, 1H, C₄-H), 7.45 (t, 2H, aromatic), 7.62 (t, 1H, aromatic), 7.95 (d, 2H, aromatic), 8.05 (s, 1H, C₈-H).

- 30 U.V. : (CH₃OH) λ_{\max} : 312nm.

Example 5.

(-) - (2R,4R) - 2-benzoyloxymethyl-4-(2'-amino-6'-cyclopropylamino-purine-9'-yl)-1,3-dioxolane (VII)



To a solution of (-)-(2R,4R)-2-benzoyloxymethyl-4-(2'-amino-6'-chloro-purine-9'-yl)-1,3-dioxolane (VIa) (600 mg) in ethanol (30 mL) was added cyclopropylamine (2 mL, = 18 eq.). The mixture was gently heated at reflux (80-85°C) for 18 h and cooled to room temperature. Solvent was evaporated to dryness in vacuo. The residue was dissolved in 100 mL of methylene chloride, washed with saturated NaHCO₃ solution, water, brine and dried over MgSO₄. Solvent was removed in vacuo and residue was purified on silica gel using EtOAc : MeOH as eluant to give the desired product (VII) as a foam in 80% yield. (506 mg).

R_f : 0.26 (CH₂Cl₂ : MeOH 95 : 5)

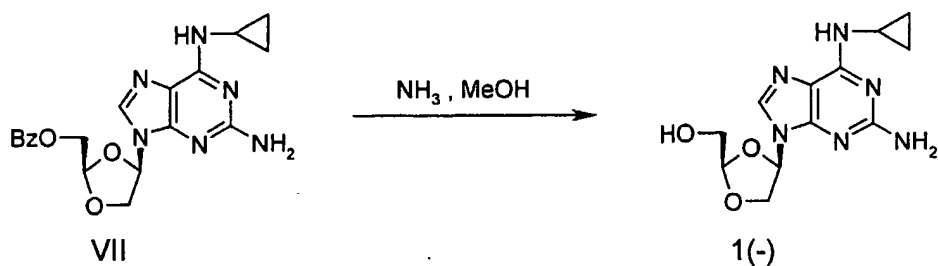
[α_D] -67.7° (c 0.2565 in CH₂Cl₂)

¹H-NMR (CDCl₃) : δ(ppm) : 0.64-0.68 (m, 2H, CH₂ of cyclopropyl), 0.91-0.96 (m, 2H, CH₂ of cyclopropyl), 3.06 (b, 1H, CH of cyclopropyl), 4.27-4.30 (dd, 1H, C₅-H), 4.54-4.57 (dd, 1H ; C₅-H) 4.60 (t, 2H, C₂-CH₂-OBz), 5.37 (b, 2H, NH₂), 5.42 (t, 1H, C₂-H, J=3.5Hz), 6.28 (b, 1H, NH), 6.35 (dd, 1H, C₄-H), 7.45 (t, 2H, aromatic), 7.58 (t, 1H, aromatic), 7.77 (s, 1H, C₈-8), 8.01(d, 2H, aromatic),

U.V. : (CH₃OH) λ_{max} : 283 and 260nm.

Example 6.

(-)-(2R,4R)-2-hydroxymethyl-4-(2'-amino-6'-cyclopropylamino-purine-9'-yl)-1,3-dioxolane (compound 1(-))



A solution of (-)-(2R,4R)-2-benzoyloxymethyl-4-(2'-amino-6'-cyclopropylamino-purine-9'-yl)-1,3-dioxolane (VII) (480 mg) in 30 mL of saturated methanolic ammonia was stirred at room temperature for 18 h. The mixture was evaporated to dryness in vacuo. The residue was dissolved in 20 mL of water, washed twice with 10 mL of methylene chloride and lyophilized to give 283 mg of white solid in 80% yield (1(-)).

R_f : 0.26 (CH_2Cl_2 : MeOH 9 : 1)

$[\alpha_D]$ -35.9° (c 0.334 in MeOH)

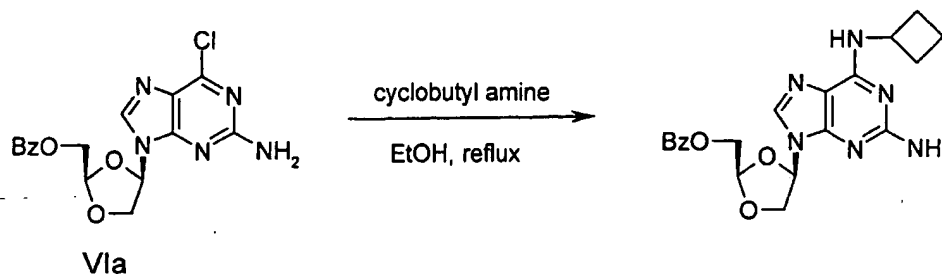
$^1\text{H-NMR}$ ($\text{DMSO}-d_6$) : δ (ppm) : 0.55 (m, 2H, CH_2 of cyclopropyl), 0.95 (m, 2H, CH_2 of cyclopropyl), 3.15 (b, 1H, CH of cyclopropyl), 3.80 (m, 2H, CH_2OH), 4.30 (dd, 1H, $\text{C}_5\text{-H}$), 4.55 (dd, 1H ; $\text{C}_5\text{-H}$), 5.08 (t, 1H, $\text{C}_2\text{-H}$), 5.17 (b, H, OH), 6.15 (b, 2H, NH_2), 6.52 (dd, 1H, $\text{C}_4\text{-H}$), 7.72 (b, 1H, NH), 8.12 (s, 1H, $\text{C}_8\text{'-8}$).

U.V. : (CH_3OH) λ_{max} : 283 and 260nm.

20

Example 7.

(-)-(2R,4R)-2-benzoyloxymethyl-4-(2'-amino-6'-cyclobutylamino-purine-9'-yl)-1,3-dioxolane



To a solution of (-)-(2R,4R)-2-benzoyloxymethyl-4-(2'-amino-6'-chloro-purine-9'-yl)-1,3-dioxolane (VIa) (250 mg) in ethanol (25 ml) was added cyclobutylamine (0.17 mL, = 3 eq.). The mixture was gently heated at reflux (80-85°C) for 18 h and cooled to room temperature. Solvent was evaporated to dryness in vacuo. The residue was dissolved in 100 mL of methylene chloride, washed with saturated NaHCO₃ solution, water, brine and dried over MgSO₄. Solvent was removed in vacuo and residue was purified on silica gel using EtOAc : MeOH 95 : 5 as eluant to give the desired product as a foam in 84% yield. (230 mg).

R_f : 0.31 (CH₂Cl₂ : MeOH 95 : 5)

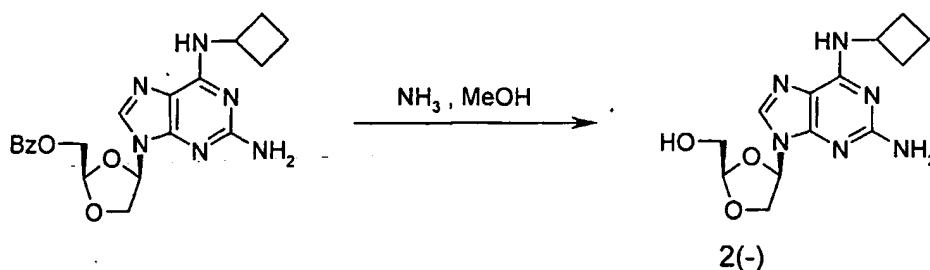
[α_D] -62.5° (c 0.4925 in CH₂Cl₂)

¹H-NMR (CDCl₃) : δ(ppm) : 1.74-1.78 (m, 2H, CH₂ of cyclobutyl), 1.95-2.00 (m, 2H, CH₂ of cyclobutyl), 2.43-2.45 (m, 2H, CH₂ of cyclobutyl), 4.27-4.30 (dd, 1H, C₅-H), 4.54-4.57 (dd, 1H ; C₅-H), 4.59 (t, 2H, C₂-CH₂-OBz), 4.75 (b, 1H, CH of cyclobutyl), 5.37 (b, 2H, NH₂), 5.41 (t, 1H, C₂-H, J=3.6Hz), 6.00 (b, 1H, NH), 6.35 (dd, 1H, C₄-H), 7.45 (t, 2H, aromatic), 7.58 (t, 1H, aromatic), 7.75 (s, 1H, C₈-H), 8.01(d, 2H, aromatic),

U.V. : (CH₃OH) λ_{max} : 283 and 263nm.

Example 8.

(-)-(2R,4R)-2-hydroxymethyl-4-(2'-amino-6'-cyclobutylamino-purine-9'-yl)-1,3-dioxolane (compound 2(-))



A solution of (-)-(2R,4R)-2-benzoyloxymethyl-4-(2'-amino-6'-cyclobutylamino-purine-9'-yl)-1,3-dioxolane (214 mg) in 20 mL of saturated methanolic ammonia was stirred at room temperature for 18 h. The mixture was evaporated to dryness in vacuo. The residue was dissolved in 20 mL of water, washed twice with 10 mL of ether and evaporated to dryness by coevaporation with ethanol to give 154 mg of pure product (2(-)) as a foam in 96% yield.

R_f : 0.52 (CH_2Cl_2 : MeOH 9 : 1)

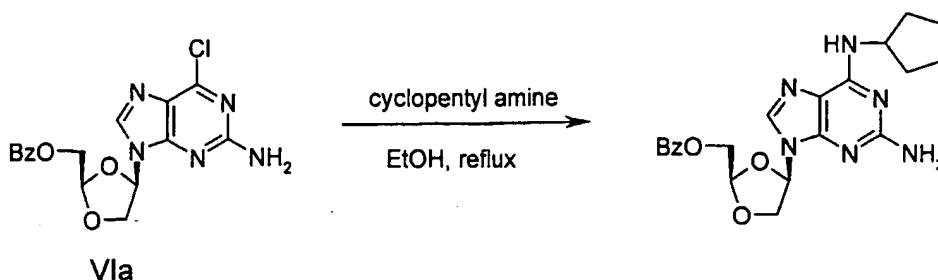
$[\alpha_D]$ -29.04° (c 0.396 in MeOH)

$^1\text{H-NMR}$ ($\text{DMSO}-d_6$) : δ (ppm) : 1.61 (m, 2H, CH_2 of cyclobutyl), 2.06 (m, 2H, CH_2 of cyclobutyl), 2.18 (m, 2H, CH_2 of cyclobutyl), 3.58 (m, 2H, CH_2OH), 4.17 (dd, 1H, $\text{C}_5\text{-H}$), 4.40 (dd, 1H ; $\text{C}_5\text{-H}$), 4.90 (b, 1H, CH of cyclobutyl), 5.01 (t, 1H, $\text{C}_2\text{-H}$), 5.42 (b, H, OH), 5.87 (b, 2H, NH_2), 6.19 (dd, 1H, $\text{C}_4\text{-H}$), 7.62 (b, 1H, NH), 7.85 (s, 1H, $\text{C}_8\text{-H}$).

U.V. : (CH_3OH) λ_{max} : 283 and 260nm.

Example 9.

(-)-(2R,4R)-2-benzoyloxymethyl-4-(2'-amino-6'-cyclopentylamino-purine-9'-yl)-1,3-dioxolane



To a solution of (-)-(2R,4R)-2-benzoyloxymethyl-4-(2'-amino-6'-chloro-purine-9'-yl)-1,3-dioxolane (VIa) (250 mg) in ethanol (15 mL) was added cyclopentylamine (0.2 mL, = 3 eq.). The mixture was gently heated at reflux (80-85°C) for 18 h and cooled to room temperature. Solvent was evaporated to dryness in vacuo. The residue was dissolved in 100 mL of methylene chloride, washed with saturated NaHCO₃ solution, water, brine and dried over MgSO₄. Solvent was removed in vacuo and residue was purified on silica gel using EtOAc and EtOAc : MeOH 95 : 5 as eluant to give the desired product as a foam in 70% yield. (191 mg).

R_f : 0.30 (CH₂Cl₂ : MeOH 95 : 5)

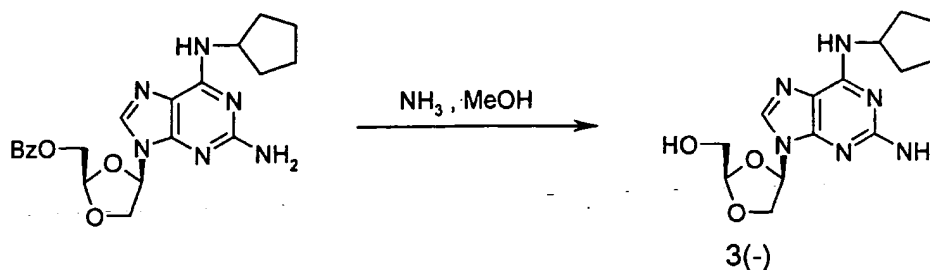
[α_D] -67.7° (c 0.363 in CH₂Cl₂)

¹H-NMR (CDCl₃) : δ(ppm) : 1.53 (m, 2H, CH₂ of cyclopentyl), 1.68 (m, 2H, CH₂ of cyclopentyl), 1.76 (m, 2H, CH₂ of cyclopentyl), 2.10 (m, 2H, CH₂ of cyclopentyl), 4.25 (dd, 1H, C₅-H), 4.54-4.54-4.60 (m, 4H ; C₅-H, C₂-CH₂-OBz and CH of cyclopentyl), 4.98 (b, 2H, NH₂), 5.42 (t, 1H, C₂-H, J=3.5Hz), 5.75 (b, 1H, NH), 6.35 (d, 1H, C₄-H), 7.45 (t, 2H, aromatic), 7.58 (t, 1H, aromatic), 7.74 (s, 1H, C₈-H), 8.01(d, 2H, aromatic),

U.V. : (CH₃OH) λ_{max} : 283 and 260nm.

Example 10.

(-)-(2R,4R)-2-hydroxymethyl-4-(2'-amino-6'-cyclopentylamino-purine-9'-yl)-1,3-dioxolane (compound 3(-))



A solution of (-)-(2R,4R)-2-benzoyloxymethyl-4-(2'-amino-6'-cyclopentylamino-purine-9'-yl)-1,3-dioxolane (180 mg) in 20 mL of saturated methanolic ammonia was stirred at room temperature for 18 h. The mixture was evaporated to dryness in vacuo. The residue was purified on silica gel using CH_2Cl_2 : MeOH 95 : 5 as eluant to give 130mg of white solid (3(-)) in 95% yield.

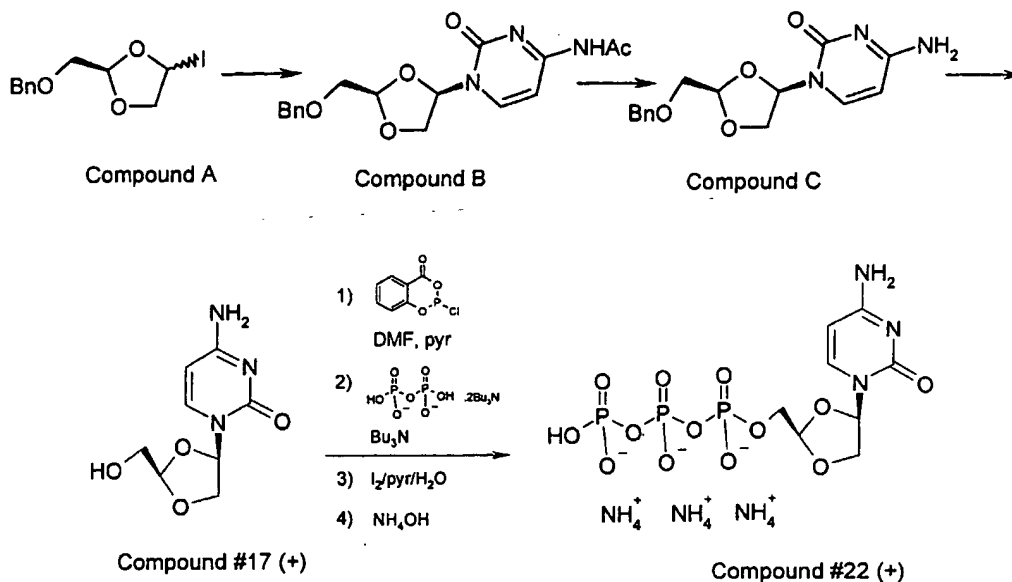
10 R_f : 0.58 (CH_2Cl_2 : MeOH 9 : 1)
 $[\alpha_D] -30.2^\circ$ (c 0.4275 in MeOH)

$^1\text{H-NMR}$ ($\text{DMSO}-d_6$) : δ (ppm) : 1.55 (m, 4H, CH_2 of cyclopentyl), 1.68 (m, 2H, CH_2 of cyclopentyl), 1.88 (m, 2H, CH_2 of cyclopentyl), 3.58 (m, 2H, CH_2OH), 4.17 (dd, 1H, $\text{C}_5\text{-H}$), 4.41 (dd, 1H ; $\text{C}_5\text{-H}$), 4.5 (b, 1H, CH of cyclopentyl), 5.01 (t, 1H, $\text{C}_2\text{-H}$), 5.14 (b, H, OH), 5.85 (b, 2H, NH_2), 6.18 (dd, 1H, $\text{C}_4\text{-H}$), 7.12 (b, 1H, NH), 7.84 (s, 1H, $\text{C}_8\text{'-H}$).

U.V. : (CH_3OH) λ_{max} : 283 and 260nm.

20 Example 11. Preparation of (+)-cis-2-hydroxymethyl-4-(cytosin-1'-yl)-1,3-dioxolane (β -D-OddC) (compound#17(+)) and (+)-triphosphate-cis-2-hydroxymethyl-4-(cytosin-1'-yl)-1,3-dioxolane (β -D-OddC-tp) (compounds #22(+)) _____

Scheme 2.



To a stirring suspension of 4-amino-1-(2-(R)-hydroxymethyl-[1,3]dioxolan-4-(R)-yl)-1H-pyrimidin-2-one (17(+)) (18.8 mg, 0.088 mmol) in dry DMF (0.49 mL) was added dry pyridine (0.13 mL) followed by a freshly prepared solution of 2-chloro-4 H-1,3,2-benzodioxaphosphorin-4-one 1.0 M in 1,4-dioxane (81 μ L, 0.081 mmol). The mixture was stirred 15 minutes at room temperature, then tributylamine (53 μ L, 0.22 mmol) and a solution of tributylammonium pyrophosphate 0.42 M in DMF (175 μ L, 0.073 mmol) were added simultaneously. The mixture was stirred another 15 minutes. A solution of I2 1% in pyridine/H₂O (98 : 2) (1.4 mL, 0.115 mmol of I) was added and the mixture was stirred 15 minutes. The excess of iodine was destroyed by adding 0.5 mL of aqueous sodium bisulfite 5%. The mixture was stirred 15 minutes, then it was concentrated under reduced pressure to remove all solvents. The residue was dissolved in water, washed two times with methylene chloride and once with ethyl acetate. The aqueous layer was concentrated and purified by charcoal column as follow: about 400 mg of charcoal, placed over a

thin layer of Celite in a funnel with fritted disk, was prewashed by passing methanol, then water (by vacuum). The crude residue was diluted in a minimum of water, acidified to pH 1-2 by adding few drops of HCl 1N, then placed on the top of the charcoal column. The column was eluted with water (25 mL) in order to remove inorganic salts, then 0.5 N ammonia (10 mL) to collect the desired triphosphate. The collected triphosphate was lyophilized, and it was then purified again on a small pad of charcoal, this time eluting only with water. The desired triphosphate comes out fast. Few fractions were collected and lyophilized to give the triphosphate ammonium salt (22(+)) , as a yellow solid (4.8 mg, 13% yield).

¹H NMR (400 MHz, D₂O) δ ppm: 7.93 (d, 1 H, 7.47 Hz), 6.21 (s, 1 H), 6.02 (d, 1 H, 7.47 Hz), 5.19 (s, 1 H), 4.20 (m, 4 H).

³¹P NMR (162 MHz, D₂O) δ ppm: -6.1 (d, 19 Hz), -10.5 (d, 19 Hz), -21.7 (t, 19 Hz).

- 20 The compounds of the present invention can be prepared by methods well known in the art. For example, such methods are described in the following references: US patent 5,041,449, PCT publication WO 92/20669 (PCT application PCT/CA92/00211), Journal of Chromatography, 645 (1993) 107-114, Tetrahedron Assymetry Vol. 4 No. 11 pp.2319-2322 (1993), Tetrahedron Assymetry Vol. 4 No. 2 pp.211-214 (1993), Bioorganic & Medicinal Chemistry Vol.3 No.8, pp.1543-1546 (1993), Tetrahedron Letters, Vol.33, No. 46, pp 6949-6952, (1992), J.Org. Chem., 34(6), pp.1547-1550 (1969),
- 30 J.Org. Chem., 52(9), pp.1794-1801 (1987), J.Am.Chem.Soc., 87(8), pp.1785-1788 (1965), J.Org. Chem. (1989), 54, pp.631-635 which are all incorporated by reference.

In a similar manner, the following compounds were synthesized:

(-) *Cis*-2-hydroxymethyl-4-(5'-cytosin-1'-yl)-1,3-Dioxolane
(Compound #17 (-))

(+) *Cis*-2-hydroxymethyl-4-(5'-cytosin-1'-yl)-1,3-Dioxolane
(Compound #17 (+))

(+) *Cis*-2-hydroxymethyl-4-(5'-fluorocytosin-1'-yl)-1,3-Dioxolane (Compound#18(+))

10 (-) *Cis*-2-hydroxymethyl-4-(5'-fluorocytosin-1'-yl)-1,3-Dioxolane (Compound#18 (-))

(+) *Cis*-2-hydroxymethyl-4-(5'-azacytosin-1'-yl)-1,3-Dioxolane
(Compound#19 (+))

(-) *Cis*-2-hydroxymethyl-4-(5'-azacytosin-1'-yl)-1,3-Dioxolane
(Compound#19 (-))

(-) -*cis*-2-hydroxymethyl-4-(5'-methylcytosin-1'-yl)-1,3-dioxolane (β -L-) (compound#20 (-)) .

(+) -*cis*-2-hydroxymethyl-4-(5'-methylcytosin-1'-yl)-1,3-dioxolane (compound#20 (+)) .

(-) -*cis*-2-hydroxymethyl-4-(N-1'-thyminy1)-1,3-dioxolane
(compound#21 (-)) .

(+) -*cis*-2-hydroxymethyl-4-(N-1'-thyminy1)-1,3-dioxolane
(compound#21 (+))

30

**Example 12 Evaluation of Nucleoside Triphosphate Analogues
In The HCV RNA-Dependent RNA Polymerase Assay**

The following references which are referenced in the example are all incorporated by reference:

1. Behrens, S., Tomei, L., De Francesco, R. (1996) *EMBO* 15, pp.12-22
2. Harlow, E, and Lane, D. (1988) *Antibodies: A Laboratory Manual*. Cold Spring Harbord Laboratory. Cold Spring Harbord. NY.
3. Lohmann, V., Körner, F., Herian, U., and Bartenschlager, R. (1997) *J. Virol.* 71, pp.8416-8428

10

Compounds were evaluated using an *in vitro* polymerase assay containing purified recombinant HCV RNA-dependent RNA polymerase (NS5B protein). HCV NS5B was expressed in insect cells using a recombinant baculovirus as vector. The experimental procedures used for the cloning, expression and purification of the HCV NS5B protein are described bellow. Follows, are details of the RNA-dependent RNA polymerase assays used to test the compounds.

20 **Expression of the HCV NS5B protein in insect cells:**

The cDNA encoding the entire NS5B protein of HCV-Bk strain, genotype 1b, was amplified by PCR using a plasmid containing a cDNA version of the full-length HCV genome as template. The oligonucleotides used to amplify this HCV region were designed to introduce a *NheI* site followed by an ATG at the 5' end of the NS5B coding region as well as a *BamHI* site at the 3' end immediately downstream of the translation stop codon. The amplified sequence, of 1.8 kb, was digested with *NheI* and *BamHI* and ligated to a predigested pBlueBacII plasmid (Invitrogen). The resulting recombinant plasmid was designated pBac/NS5B. Sf9 cells were co-transfected with 3 µg of pBac/NS5B, together with 1 µg of linearized baculovirus DNA (Invitrogen), as described in the manufacturer's protocol. Following two rounds of plaque purification, an NS5B-recombinant baculovirus, BacNS5B, was

30

isolated. The presence of the recombinant NS5B protein was determined by western blot analysis (Harlow and Lane, 1988) of BacNS5B-infected Sf9 cells, using a HCV NS5B specific rabbit polyclonal antiserum (anti-NS5B). Infections of Sf9 cells with this plaque purified virus were performed in one-liter spinner flasks at a cell density of 1.2×10^6 cells/ml and a multiplicity of infection of 5.

Preparation of a soluble recombinant NS5B protein :

- 10 Sf9 cells were infected as described above. Sixty hours post-infection, cells were harvested then washed twice with phosphate buffer saline (PBS). Total proteins were solubilized as described in Lohmann et al. (1997) with some modifications. In brief, proteins were extracted in three steps, S1, S2, S3, using lysis buffers (LB) I, LB II and LB III (Lohmann et al, 1997). The composition of LBII was modified to contain 0.1 % triton X-100 and 150 mM NaCl to reduce the amount of solubilized NS5B protein at this step. In addition, sonication of cell extracts was avoided
- 20 throughout the protocol to preserve the integrity of the protein structure.

Purification of recombinant NS5B using fast protein liquid chromatography (FPLC) :

- Soluble NS5B protein in the S3 fraction was diluted to lower the NaCl concentration to 300 mM, then it incubated batchwise with DEAE sepharose beads (Amersham-Pharmacia) for 2 hrs at 4°C, as described by Behrens et al. (1996). Unbound material was cleared by centrifugation for 15 min at
- 30 4°C, at 25 000 rpm using a SW41 rotor (Beckman). The supernatant was further diluted to lower the NaCl concentration to 200 mM and subsequently loaded, with a flow rate of 1 ml/min, on a 5 ml HiTrap® heparin column (Amersham-Pharmacia) connected to an FPLC® system (Amersham-Pharmacia). Bound proteins were eluted in 1 ml fractions,

- using a continuous NaCl gradient of 0.2 to 1 M, over a 25 ml volume. NS5B-containing fractions were identified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), followed by western blotting using the anti-NS5B antiserum at a dilution of 1:2000. Positive fractions were pooled and the elution buffer was exchanged against a 50 mM NaPO₄ pH 7.0, 20 % glycerol, 0.5 % triton X-100 and 10 mM DTT, using a PD-10 column (Amersham-Pharmacia). The sample was then loaded onto a 1 ml HiTrap[®] SP column (Amersham-Pharmacia), with a flow rate of 0.1 ml/min. Bound proteins were eluted using a continuous 0 to 1 M NaCl gradient over a 15 ml volume. Eluted fractions were analyzed by SDS-PAGE and western blotting. Alternatively, proteins were visualized, following SDS-PAGE, by silver staining using the Silver Stain Plus kit (BioRad) as described by the manufacturer. Positive fractions were tested for RdRp activity (see below) and the most active ones were pooled, and stored as a 40 % glycerol solution at -70°C.
- 10
- 20 **In vitro RNA-dependent RNA polymerase assays used to evaluate triphosphate form of guanosine analogues:**
- RdRp assays were conducted using either homopolymeric template/primers or in vitro transcribed RNA templates. All RdRp reactions which used homopolymeric template/primers were performed in a total volume of 50 µl of a buffer consisting of 20 mM Tris-HCl pH 7.5, 1 mM DTT, 50 mM NaCl, 0.5 mM MnCl₂, 5 µCi [γ ³²P]-GTP (3000 Ci/mmol), and 4 µM of cold GTP. Standard HCV RdRp reactions contained 200 ng of purified NS5B protein. Guanosine analogues were tested in the presence of polyC/oligoG. PolyC RNAs (Amersham-Pharmacia) was resuspended at 400 ng/µl. The primer oligoG₁₂ (MWG, Germany) was diluted to a concentration of 20 pmol/µl (7.6 ng/µl). Templates and primers were mixed volume to volume, denatured at 95°C for 5 min and annealed at 37°C for 10 min. Following a two hour incubation at 22°C, reactions
- 30

were stopped by the addition of 100 μ g of sonicated salmon sperm DNA (Life Technologies) and 1 ml of 10 % trichloroacetic acid-0.5 % tetrasodium pyrophosphate (TCA-PPI). Nucleic acids were precipitated at 4°C for 30 min after which samples were filtered on GF/C glass microfiber filters (Millipore). Membranes were subsequently washed with 25 ml of a 1% TCA-0.1 % PPI solution, then air dried. Incorporated radioactivity was quantified using a liquid scintillation counter (1450-Microbeta, Wallac).

- 10 Heteropolymeric RNA templates were generated by run-off transcription. As template for these transcription reactions, a recombinant pcDNA3 plasmid (Invitrogen) containing a cDNA version of the HCV genome was used and referred to as pcDNA/HCVfl. In vitro transcriptions were performed using the MEGAscript™ kit (Ambion), as suggested by the manufacturer. In brief, the plasmid pcDNA/HCVfl was linearized with *EcoRI* to generate a truncated HCV transcript of about 6900 nucleotides. Linearized DNA was extracted with a one to one volume of phenol/chloroform, precipitated
- 20 with ethanol, then 1 μ g of this linearized DNA was used as template in T7 RNA polymerase-driven in vitro transcription reactions. Transcripts were extracted using the TRIZOL® reagent (Life Technologies) and an aliquot (1 μ g) was used as template in RdRp assays. RdRp reactions using heteropolymeric templates were carried out under the same conditions as described above for homopolymeric template/primers, with the exception that the substrate mixture composition consisted of 5 μ Ci of [γ ³²P]-GTP, 1 μ M cold GTP and 100 μ M of the remaining nucleotides (ATP, CTP
- 30 and UTP). Reaction mixtures were subsequently processed as described above for homopolymeric template-containing reactions. Results are shown below:

Compound	HCV polymerase
	% inhibition
9(-)-triphosphate	44%@100 μ M
13(-) triphosphate	82%@50 μ M IC ₅₀ =10 μ M
14(-)-triphosphate	6%@50 μ M

In vitro RNA-dependent RNA polymerase assays used to evaluate triphosphate form of cytosine analogues:

RdRp assays were conducted using either homopolymeric template/primers or *in vitro* transcribed RNA templates.

All RdRp reactions which used homopolymeric template/primers were performed in a total volume of 50 μ l of a buffer consisting of 20 mM Tris-HCl pH 7.5, 1 mM DTT, 50 mM NaCl, 0.5 mM MnCl₂, 5 μ Ci [γ ³²P]-CTP (3000 Ci/mmol), and 5 μ M of cold CTP. Standard HCV RdRp reactions contained 200 ng of purified NS5B protein. Cytosine analogues were tested in the presence of polyrI/oligodC. PolyrI RNAs (Amersham-Pharmacia) was resuspended at 400 ng/ μ l. The primer oligodC₁₂ (Life Technologies) was diluted to a concentration of 20 pmol/ μ l. Templates and primers were mixed volume to volume, denatured at 95°C for 5 min and annealed at 37°C for 10 min. Following a two hour incubation at 22°C, reactions were stopped by the addition of 100 μ g of sonicated salmon sperm DNA (Life Technologies) and 1 ml of 10 % trichloroacetic acid-0.5 % tetrasodium pyrophosphate (TCA-PPI). Nucleic acids were precipitated at 4°C for 30 min after which samples were filtered on GF/C glass microfiber filters (Millipore). Membranes were subsequently washed with 25 ml of a 1% TCA-0.1 % PPI solution, then air dried.

Incorporated radioactivity was quantified using a liquid scintillation counter (1450-Microbeta, Wallac).

Heteropolymeric RNA templates were generated by run-off transcription. As template for these transcription reactions, a recombinant pcDNA3 plasmid (Invitrogen) containing a cDNA version of the HCV genome was used and referred to as pcDNA/HCVfl. In vitro transcriptions were performed using the MEGAscript™ kit (Ambion), as suggested by the manufacturer. In brief, the plasmid pcDNA/HCVfl was
10 linearized with *EcoRI* to generate a truncated HCV transcript of about 6900 nucleotides. Linearized DNA was extracted with a one to one volume of phenol/chloroform, precipitated with ethanol, then 1 µg of this linearized DNA was used as template in T7 RNA polymerase-driven in vitro transcription reactions. Transcripts were extracted using the TRIZOL® reagent (Life Technologies) and an aliquot (1 µg) was used as template in RdRp assays.

RdRp reactions using heteropolymeric templates were carried out under the same conditions as described above for
20 homopolymeric template/primers, with the exception that the substrate mixture composition consisted of 5 µCi of [γ ³²P]-CTP, 1 µM cold CTP and 100 µM of the remaining nucleotides (ATP, GTP and UTP). Reaction mixtures were subsequently processed as described above for homopolymeric template-containing reactions. Results are shown below:

COMPOUND	HCV polymerase
	% inhibition
COMPOUND#22(+)	85%@50µM IC ₅₀ =7.7µM

COMPOUND#22(-)	15%@100µM
COMPOUND#23(+)	57%@100µM
COMPOUND#23(-)	81%@100µM IC ₅₀ =16µM
COMPOUND#24(+)	12%@50µM
COMPOUND#24(-)	8%@100µM
COMPOUND#25(+)	9%@50µM
COMPOUND#26(+)	47%@100µM

EXAMPLE 13**Cytotoxicity Assay**

The cytotoxicity of test compounds was evaluated according to the following procedure:

10 Flat bottom 96 well plates were plated with 5X10E3 Vero-34 cells/well and 1X10E4 Hs-68 or Wi-38 cells/well respectively and incubated overnight at 37°C and 5% CO₂/air. After incubation, the supernatant medium was removed and replaced with test compound dilutions in 2% DMEM (150 µl). The cells were then incubated 48 hours in a 5% CO₂ incubator at 37°C.

50µl/well of 10µCi/ml solution of [³H]-methyl thymidine (specific activity of approx. 2Ci/mmol) was added to the culture medium and incubated overnight (18 hours) in a 5% CO₂ incubator at 37°C.

20 Cells were then collected onto a fiberglass filter (Printed Filtermat A 1450-421 Wallac) with a Tomtec cell harvester. Suspended cells were collected directly onto filter while for adherent cells, the medium was first removed, then the

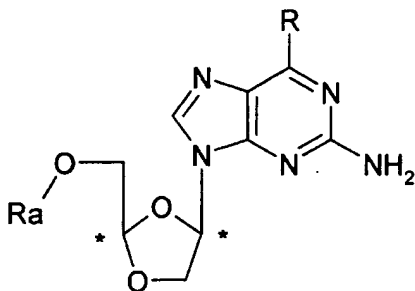
cells washed with PBS and trypsinized for 2-3 minutes (50µl trypsin/well) before collecting.

Filters were dried for 1 hour at 37-40°C and then placed into bags (1450-microbeta # 1450-432 Wallac) with 4.5 ml of Betascint and counts obtained with Microbeta 1450 Wallac.

The percent of cell proliferation was determined by comparison to the control (no test compound) and thereby
10 establishing 50% inhibitory concentration is established.

CLAIMS

1. The use of at least one compound having the formula I or a pharmaceutically acceptable salt thereof for treating or preventing a *Flaviviridae* viral infection in a host



(I)

wherein:

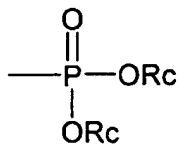
R is H, $-NR_2R_3$ or OR_4 wherein

R_2 is H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_3 -cycloalkyl;

R_3 is H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl;

R_4 is H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl; and

R_a is chosen from H, monophosphate, diphosphate, triphosphate, carbonyl substituted with a C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-10} aryl and



wherein each R_c is independently chosen from H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl and an hydroxy protecting group;

wherein said nucleoside is present in the form of the (-) enantiomer, (+) enantiomer and mixtures thereof, including racemic mixtures.

2. The use of claim 1 wherein R is NH₂, H or OH.
3. The use of claim 1 wherein R is NH₂ or OH.
4. The use of claim 1 wherein R is OH.
5. The use of claim 1 wherein R is -NR₂R₃ wherein R₂ is cyclopropyl and R₃ is H.
6. The use of claim 1 wherein R_a is chosen from H, monophosphate, diphosphate, and triphosphate.
7. The use of claim 1 wherein R_a is chosen from monophosphate, diphosphate, and triphosphate.
8. The use of claim 1 wherein R_a is triphosphate.
9. The use of claim 1 wherein R_a is H.
10. The use as defined in anyone of claims 1 to 9 wherein said compound of formula I of the present invention is substantially in the form of the (-) enantiomer.
11. The use of claim 1 wherein said compound is chosen from:

Compound 1 cis-2-hydroxymethyl-4-(2'-amino-6'-cyclopropylamino-purine-9'-yl)-1,3-dioxolane;

Compound 2 cis-2-hydroxymethyl-4-(2'-amino-6'-cyclobutylamino-purine-9'-yl)-1,3-dioxolane;

Compound 3 cis-2-hydroxymethyl-4-(2'-amino-6'-cyclopentylamino-purine-9'-yl)-1,3-dioxolane;

Compound 4 -cis-2-hydroxymethyl-4-(2'-6'-diamino-purin-9'-yl)-1,3-dioxolane;

Compound 5 cis-2-hydroxymethyl-4-(guanin-9'-yl)-1,3-dioxolane;

Compound 6 cis-2-hydroxymethyl-4-(adenin-9'-yl)-1,3-dioxolane;

Compound 7 cis-2-hydroxymethyl-4-(2'-amino-6'-chloro-purin-9'-yl)-1,3-dioxolane; or

Compound 8 cis-2-hydroxymethyl-4-(2'-amino-purin-9'-yl)-1,3-dioxolane.

12. The use of claim 1 wherein said compound is chosen from

Compound 1(-) cis-2-hydroxymethyl-4-(2'-amino-6'-cyclopropylamino-purine-9'-yl)-1,3-dioxolane;

Compound 2(-) cis-2-hydroxymethyl-4-(2'-amino-6'-cyclobutylamino-purine-9'-yl)-1,3-dioxolane;

Compound 3(-) cis-2-hydroxymethyl-4-(2'-amino-6'-cyclopentylamino-purine-9'-yl)-1,3-dioxolane;

Compound 4(-) -cis-2-hydroxymethyl-4-(2'-6'-diamino-purin-9'-yl)-1,3-dioxolane;

Compound 5(-) cis-2-hydroxymethyl-4-(guanin-9'-yl)-1,3-dioxolane;

Compound 6(-) cis-2-hydroxymethyl-4-(adenin-9'-yl)-1,3-dioxolane;

Compound 7(-) cis-2-hydroxymethyl-4-(2'-amino-6'-chloro-purin-9'-yl)-1,3-dioxolane; or

Compound 8(-) cis-2-hydroxymethyl-4-(2'-amino-purin-9'-yl)-1,3-dioxolane.

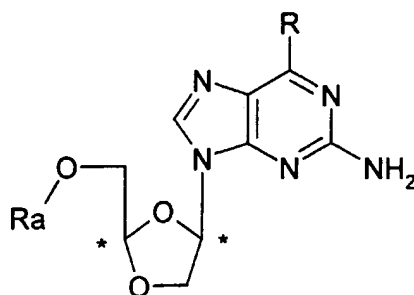
13. The use as defined in anyone of claims 1 to 12 wherein said *Flaviviridae* viral infection is hepatitis C (HCV).

14. The use of a therapeutically effective amount of at least one compound according to formula I as defined in

anyone of claims 1 to 12 and at least one further antiviral agent for treating or preventing a hepatitis C viral infection in a host.

15. The use according to claim 14 wherein the antiviral agent is chosen from viral serine protease inhibitor, viral polymerase inhibitor and viral helicase inhibitor.
16. The use according to claim 14 wherein the antiviral agent chosen from interferon (IFN), interferon α -2a, interferon α -2b, consensus interferon (CIFN), ribavirin, amantadine, rimantadine, interleukine-12, ursodeoxycholic acid (UDCA), glycyrrhizin and silybum marianum.
17. The use according to claim 14 wherein the antiviral agent chosen from interferon α and ribavirin.
18. A pharmaceutical composition for treating or preventing a hepatitis viral C infection comprising administering at least one compound according to formula I as defined in anyone of claims 1 to 12 together with at least one pharmaceutically acceptable carrier or excipient.
19. The use of a compound according to formula I as defined in anyone of claims 1 to 12 for the manufacture of a medicament for treating or preventing a viral *Flaviviridae* infection in a host.
20. The use of a therapeutically effective amount of at least one compound having the formula I or a

pharmaceutically acceptable salts thereof, for inhibiting or reducing the activity of viral polymerase in a host:



(I)

wherein:

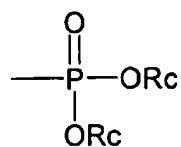
R is H, $-NR_2R_3$ or OR_4 wherein

R_2 is H, C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl;

R_3 is H or a C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl;

R_4 is H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl; and

R_a is chosen from H, monophosphate, diphosphate, triphosphate, carbonyl substituted with a C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-10} aryl and

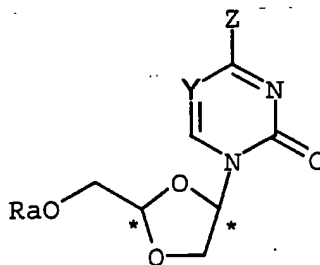


wherein each R_c is independently chosen from H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl and an hydroxy protecting group;

wherein said nucleoside is present in the form of the (-) enantiomer, (+) enantiomer and mixtures thereof, including racemic mixtures.

21. The use of claim 20 wherein said viral polymerase is a HCV polymerase.

22. The use of a therapeutically effective amount of at least one compound having the formula II or a pharmaceutically acceptable salt therefor for treating or preventing a *Flaviviridae* viral infection in a host



(II)

wherein

Z is H, $-NR'_2R_3$ or OR_4 wherein

R'_2 is H, C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl;

R_3 is H or a C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl;

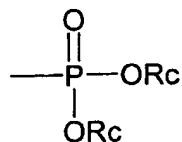
R_4 is H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl; and

Y is N or C-X;

X is chosen from of H, halogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, CN, CF_3 , N_3 , NO_2 , C_{6-10} aryl, C_{6-10} heteroaryl and COR_b

wherein R_b is chosen from of H, OH, SH, C_{1-6} alkyl, C_{1-6} aminoalkyl, C_{1-6} alkoxy and C_{1-6} thioalkyl;

and R_a is chosen from of H, monophosphate, diphosphate, triphosphate, carbonyl substituted with a C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{6-10} aryl, and



wherein each R_c are independently chosen from H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl and an hydroxy protecting group,

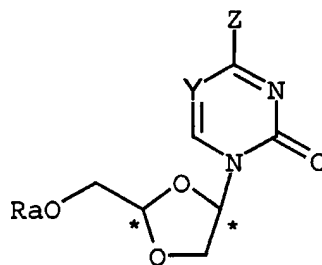
wherein said compound is in the form of a single enantiomer or a mixture thereof including racemic mixtures.

23. The use of claim 22 wherein said compound of formula II of the present invention is substantially in the form of the (+) enantiomer.
24. The use of claim 22 wherein said compound of formula II of the present invention is substantially in the form of the (-) enantiomer.
25. The use of anyone of claims claim 22 to 24 wherein Ra is chosen from H, monophosphate, diphosphate, and triphosphate.
26. The use of anyone of claims claim 22 to 24 wherein Ra is chosen from monophosphate, diphosphate, and triphosphate.
27. The use anyone of claims claim 22 to 24 wherein Ra is triphosphate.
28. The use anyone of claims claim 22 to 24 wherein Ra is H.
29. The use anyone of claims claim 22 to 24 wherein Y is C-X.
30. The use anyone of claims claim 22 to 24 wherein Y is C-X and X is H, methyl, or Halogen.
31. The use anyone of claims claim 22 to 24 wherein Y is C-X.
32. The use anyone of claims claim 22 to 24 wherein Y is C-X and X is H, methyl or halogen.

33. The use anyone of claims claim 22 to 24 wherein Y is C-X and X is chosen from of methyl, $-C(H)=CH_2$ and $-C\equiv CH$.
34. The use anyone of claims claim 22 to 24 wherein Y is C-X and X is H, methyl or halogen.
35. The use anyone of claims claim 22 to 24 wherein Y is C-X and X is H, methyl or F.
36. The use anyone of claims claim 22 to 24 wherein Y is C-X and X is H or F.
37. The use anyone of claims claim 22 to 24 wherein Y is C-X and X is H.
38. The use anyone of claims claim 22 to 24 wherein Y is C-X and X is F.
39. The use anyone of claims claim 22 to 24 wherein Z is OH.
40. The use anyone of claims claim 22 to 24 wherein Z is NH_2 .
41. The use of claim 22 wherein the compound of formula II is (+)Cis-2-hydroxymethyl-4-(cytosin-1'-yl)-1,3-Dioxolane (Compound #17(+)).
42. The use of claim 22 wherein the compound of formula II is (+)Cis-2-hydroxymethyl-4-(5'-fluorocytosin-1'-yl)-1,3-Dioxolane (Compound #18(+)).
43. The use of claim 22 wherein the compound of formula II is (-)Cis-2-hydroxymethyl-4-(5'-fluorocytosin-1'-yl)-1,3-Dioxolane (Compound #18(-)).

44. The use of claim 22 wherein the compound of formula II is (+)Cis-2-hydroxymethyl-4-(5'-azacytosin-1'-yl)-1,3-Dioxolane (Compound #19(+)).
45. The use of claim 22 wherein the compound of formula II is (+)-cis-2-hydroxymethyl-4-(5'-methylcytosin-1'-yl)-1,3-dioxolane(β -D-)(compound#20) (+) .
46. The use of claim 22 wherein the compound of formula II is (+)-cis-2-hydroxymethyl-4-(N-1'-thiminy1)-1,3-dioxolane (-D-)(compound#21) (+) .
47. The use of claims 22 to 46 wherein said Flaviviridea viral infection is hepatitis C.
48. The use a therapeutically effective amount of at least one compound according to formula II as defined in anyone of claims 22 to 46 and at least one further antiviral agent for treating or preventing a hepatitis C viral infection in a host.
49. The use according to claim 48 wherein the further antiviral agent is chosen from of viral serine protease inhibitor, viral polymerase inhibitor and viral helicase inhibitor.
50. The use according to claim 48 wherein the antiviral agent is chosen from interferon (IFN), interferon α -2a, interferon α -2b, consensus interferon (CIFN), ribavirin, amantadine, rimantadine, interleukine-12, ursodeoxycholic acid (UDCA), glycyrrhizin and silybum marianum.

51. The use according to claim 48 wherein the antiviral agent chosen from interferon α and ribavirin.
52. A pharmaceutical composition for treating or preventing a hepatitis viral C infection comprising at least one compound according to formula II as defined in anyone of claims 20 to 46 together with at least one pharmaceutically acceptable carrier or excipient.
53. The use of a compound according to formula II as defined in anyone of claims 22 to 46 for the manufacture of a medicament for treating or preventing a viral *Flaviviridae* infection in a host.
54. The use of a therapeutically effective amount of at least one compound having the formula II or a pharmaceutically acceptable salt thereof for inhibiting or reducing the activity of viral polymerase in a host:



(II)

wherein

Z is H, $-NR_2R_3$ or OR_4 , wherein

R_2 is H, C_{1-6} alkyl, C_{2-6} alkenyl, or C_{2-6} alkynyl;

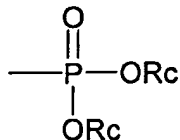
R_3 is H or a C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl;

R_4 is H, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl; and

Y is N or C-X;

X is chosen from of H, halogen, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, CN, CF₃, N₃, NO₂, C₆₋₁₀ aryl, C₆₋₁₀ heteroaryl and COR_b wherein **R_b** is chosen from of H, OH, SH, C₁₋₆ alkyl, C₁₋₆ aminoalkyl, C₁₋₆ alkoxy and C₁₋₆ thioalkyl;

and **R_a** is chosen from of H, monophosphate, diphosphate, triphosphate, carbonyl substituted with a C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl, C₆₋₁₀ aryl, and



wherein each **R_c** are independently chosen from H, C₁₋₆ alkyl, C₂₋₆ alkenyl, C₂₋₆ alkynyl and an hydroxy protecting group,

wherein said compound is in the form of a single enantiomer or a mixture thereof including racemic mixtures.

55. The use of claim 54 wherein said viral polymerase is a HCV polymerase

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
10 May 2001 (10.05.2001)

PCT

(10) International Publication Number
WO 01/32153 A3

(51) International Patent Classification⁷: **A61K 31/505**,
31/52, 31/53, A61P 31/14

(21) International Application Number: PCT/CA00/01316

(22) International Filing Date:
3 November 2000 (03.11.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/163,405 4 November 1999 (04.11.1999) US
60/163,394 4 November 1999 (04.11.1999) US

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(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

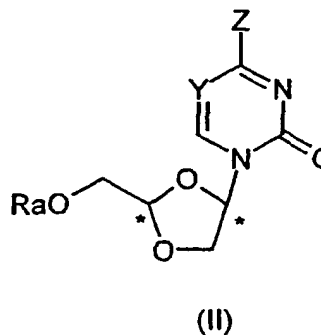
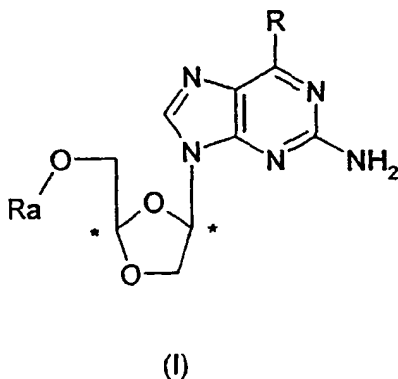
Published:

— with international search report

(88) Date of publication of the international search report:
1 November 2001

For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: METHOD FOR THE TREATMENT OR PREVENTION OF *FLAVIVIRIDAE* VIRAL INFECTION USING NUCLEO-
SIDE ANALOGUES



(57) Abstract: In accordance with the present invention there is provided a method for treating or preventing a *Flaviviridae* viral infection in a host comprising administering a therapeutically effective amount of at least one compound of formula (I) or (II) or a pharmaceutically acceptable salt thereof, wherein Ra, R, Z and Y are defined in the application.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/01316

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 A61K31/505 A61K31/52 A61K31/53 A61P31/14		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 7 A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, WPI Data, PAJ, CHEM ABS Data, BIOSIS, EMBASE, MEDLINE		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	formula (L) page 5, line 31 - line 41	1-55
-/--		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents : *A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *&* document member of the same patent family		
Date of the actual completion of the international search 23 April 2001		Date of mailing of the international search report 11/05/2001
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl. Fax: (+31-70) 340-3016		Authorized officer Villa Riva, A

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 00/01316

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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